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# PARASITOLOGY

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# PARASITOLOGY

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BRITISH MUSEUM NATURAL HISTORY

# THE LIFE HISTORY OF *DISTOMA LUTEUM* N. SP. WITH NOTES ON SOME CERCARIAE AND REDIAE FOUND IN S. AFRICA.

BY J. D. F. GILCHRIST, M.A., D.SC., PH.D.

SOME years ago, in endeavouring to obtain various stages in the life history of the liver-fluke in South Africa for teaching purposes, I found that, although the parasite is fairly common on some farms in this country, the intermediate host had not yet been definitely ascertained. In Europe, as is well known, the intermediate host is the fresh-water snail *Limnaea truncatula*, but this species has not been recorded with certainty from South Africa. The commonest fresh-water snail here is *Physa* (*Isidora*) *tropica* and a preliminary examination of a few specimens of this mollusc from Muizenberg Lake revealed abundant stages of fluke very closely resembling those of the liver-fluke. Additional specimens procured from the neighbourhood also afforded abundant material of the same stages. The only difficulty in accepting this as a solution of the problem was that, though placed in the most favourable conditions, the free swimming form (cercaria) was never observed to encyst.

I called attention in the *Agricultural Journal of the Cape of Good Hope*, Dec. 1910, to the occurrence of this fluke-like stage and requested the assistance of South African farmers in following up the investigation. Some replies were received describing the serious damage done to sheep by the liver-fluke, but no further light was thrown on the subject. One correspondent forwarded a specimen of *Achatina zebra* as the possible host, but as this is not a water snail, it is very improbable that it harbours the intermediate stage of the fluke; another stated that he had found the intermediate stage in a worm in which he had actually seen the free swimming form developing and escaping into the water. This most interesting observation however again proved a false trail for the "worms" proved to be the worm-like strings of frogs' eggs in which the tadpole, developing and escaping into the water, had been observed!

In addition to the Cercaria found in *Physa*, I noted also in the *Agricultural Journal* the occurrence of three other kinds, found in the course of the enquiry, viz., a large somewhat flat cercaria with a bifurcate tail, a cercaria with eyes (both found also in *Physa*), and a small cercaria, with a comparatively long tail provided with numerous bristles, found in a small snail, *Tomichia ventricosa*.

It may point to something worthy of further attention that, though in America and other countries the fluke is abundant, the intermediate host does not seem to have been determined with complete certainty. Several molluscs are suspected as the probable host, yet it should apparently be easy to demonstrate this. In Australia it is of interest to note that the intermediate host of the liver-fluke is believed to be a species of *Physa*, in which rediae and cercariae were found resembling those of the liver-fluke, but that the same difficulty was encountered as in South Africa in finding the encysted stage, though this was not regarded as a serious objection to accepting the rediae and cercariae as those of the fluke.

Renewed attempts to find the missing intermediate stage in *C. comma* resulted in the discovery that the cercariae, after swimming about freely, returned again to a snail, entered by the nephridial opening and became encysted in the region of the pericardium. Obviously the other host therefore was some animal that lived on the snails. The numerous frogs, which inhabited the same water in which this snail occurs, were suspected, and experiments showed conclusively that they were responsible for the adult fluke of this cercaria. This fluke is abundant in the alimentary canal of frogs, and is closely allied to *Distoma echinatum* Zeder., but, as its life history shows, it is a different species and may be called *D. luteum* from its characteristic colour.

The facility with which all the stages of this fluke can be found renders it a useful type for students of zoology in South Africa.

Search was then made elsewhere for the intermediate stages of the liver-fluke. At one time the sheep on certain lands at the Agricultural College at Stellenbosch were badly infected with fluke. No sheep were in consequence allowed on these grounds, and no signs of the disease were subsequently found in the locality. *Physa tropica* occurred in abundance here but on dissection did not reveal any rediae or cercariae. There had been no cases of the disease for several years here, but I was informed by Mr C. P. Lounsbury that a case of fluke in a horse had occurred not far off at a place, Koelenhof, a few miles distant. This place we visited and specimens of the ubiquitous *Physa tropica* were readily procured, but no other fresh-water snail. Nothing new was expected from these, but specimens were collected in a tube, in which shortly afterwards several cercariae were found swimming about, obviously quite different from those previously procured from the same species of snail, as they were of a very dark, almost black colour. This was not the only difference, for by the time Cape Town was again reached, several had encysted on the sides of the glass tube, the cysts being of the same black colour. This was interesting, but obviously did not throw light on the liver-fluke, as its cercariae and cysts are not black. This cercaria had eyes, and is, without much doubt, the free swimming stage of the cercaria previously noted in the *Agricultural Journal*.

The method of formation of the cyst is of interest in this form. The whole process could readily be observed. The animal settled down with violent wriggling of the tail, which ultimately became detached. Meanwhile the body extruded a great number of short dark rhabdite-like bodies. These were applied, the one to the other, till a dome-like structure was formed over the body. At the periphery this extended over the substratum so that a very firm attachment was secured.

Some time later (1911) on a visit to Natal some fresh-water snails were procured with the assistance of Mr Burnup, a recognised authority on South African shells, and Dr Warren kindly placed his laboratory at my disposal for their examination. A large redia was found in *Limnaea natalensis*, but the corresponding cercaria was not found. The cercaria may be *C. obscura* of Sonsino who found it in *Limnaea natalensis*. *C. obscura* was about 1 mm. long and developed from rediae of 2 mm.

Several specimens of *Physopsis africana* with characteristic cercaria of the bifurcated-tail type were also found, but no traces of stages anything like those of the liver-fluke. Another characteristic cercaria was found at the same time, it was oblong measured about 0·3 × 0·05 mm. and had a clear rod-like spine about 0·01 mm. long anteriorly. The tail was comparatively short. Their rediae were short, thick and colourless.

In discussing the problem of the liver-fluke, Mr Burnup mentioned that some shells resembling *L. truncatula* had been collected by Mr J. McBean at Pretoria, and were now in the Burnup Collection in the Pietermaritzburg Museum. These were compared carefully with specimens of this shell from Madeira, in the same collection, and in Mr Burnup's opinion, they seemed to be identical. For the following records of *L. truncatula* in South Africa I am indebted to Mr Burnup.

1899. *Limnaea umlaasiana* Küst. = (fide Bourgt.) *L. truncatula* Drap (sic) *Ann. and Mag. Nat. Hist.* ser. 7, vol. IV. p. 112 (Natal).

1898. *Limnaea truncatula*, Müll., M. and P. (Natal) *Proc. Malac. Soc.* vol. III. p. 184. Melvill and Ponsonby place it in a list of species introduced into Natal.

1898. *Limnaeus umlaasianus*, Küst. = (fide Bourgt.) *L. truncatula*, Mllv. Sturany, *Südafr. Land und Sussw. Mollusken*, p. 74 (Natal).

1874. *Limnaea truncatula* Müll.? (Tunis, Algiers, Abyssinia). C. F. Jickali, *Fauna d. Land- und Süssw. Moll.* N.O. Afr. p. 194.

It may therefore be that *L. truncatula* does occur in South Africa, though not in great abundance, and if so, this would be a sufficient explanation of the presence of the disease here. It may be objected that it is very unlikely that a fresh-water snail could have been imported from Europe to South Africa, as the animal itself will not live long out of fresh water, and its eggs are readily dried up. A possible explanation may however be found in the fact that European carp were known to have been introduced into South Africa at an early time, and it is not improbable that fresh-water plants and snails were introduced with them, to keep the water fresh and to supply food. There is certainly no doubt that the snails could have been introduced at a later date, as even the South African Governments have introduced, not only fresh-water fish, but also plants and snails from Europe, and may thus be unwittingly keeping up a supply of the dreaded liver-fluke. The solution of the problem has therefore an important practical side.

It not infrequently happens that, in the unsuccessful search for one thing, other new facts of equal interest are disclosed, and in this case though the liver-fluke question has not been cleared up, the occurrence of so many different cercariae is of itself of great interest and opens up a field for investigation of life histories for the student of Zoology in South Africa.

## LIFE HISTORY OF *DISTOMA LUTEUM*.

About the beginning of the summer months (Oct. 1910) a number of *Physa tropica* from Muizenberg Lake were dissected, and about five per cent. were found to be infected with rediae and cercariae. At a later date additional specimens were procured, and from these there appeared in the collecting tubes a number of free swimming cercariae. When dissected a large proportion were found to be infected. Other lots were from time to time examined, and about the end of January all the specimens of the snail procured were, with very few exceptions, badly infected with the parasite. Meanwhile the lake had been rapidly drying up, and the snails were then found at the roots of water plants, or in the muddy and grassy bottom. Only one or two per cent. of the snails had a healthy liver, unattacked by parasites. The infection however did not seem to affect their reproductive powers, as numerous clusters of 20 to 30 eggs were deposited on objects in the vessel, in which the snails were kept. Later on all snails found proved to be infected, and towards the end of February and beginning of March, the lake dried up, and no more snails were to be found even in places with water.

*Redia.* The smallest free redia had a well developed muscular pharynx, an anterior ring,. sometimes assuming the appearance of four blunt projections, and two prominent posterior projections. One was observed to escape from the parent redia (about three times its size) at a point behind the anterior collar. Another free redia, of about the same size as this small one, had the alimentary canal well filled with food. The full grown rediae were of a brick-red colour.

*Cercaria.* The mature cercariae are clear and transparent by transmitted light, white by reflected light. They are often observed to be very active within the rediae, twisting about and passing from one end to the other. When they escaped from the redia they were well developed with a flattened body. The outline of the body varied much according to its active movements.

The cercariae, when they have escaped from the body of the snail and are free in the water, are very active, and keep the body flexed so as to assume an appearance like a comma or point of interrogation. They measured in the living state when stretched out 0·19 by 0·17 mm. Another, circular in form, measured 0·18 mm. in diameter. These measurements it may be noted are much less than those of the cercaria of *Distoma hepaticum,* which are given as 0·28 by 0·23 mm. The posterior sucker is frequently thrust out to a considerable extent, even occasionally assuming a pedunculate appearance. At each side of the anterior end of the body there was a slight projection or shoulder, but no spines were seen either on the head or body. It differed in this respect from the cercaria of *D. echinatum.* These free cercariae were afterwards procured in thousands by keeping 20 or 30 snails in a dish of water for a few hours. At first they crowded towards the light, but later on swarmed towards the spot furthest from it. This movement was very decided and comparatively rapid, about 6 inches in a minute. They did not seem to be attracted by any grass or water weeds placed in the jar, but it was noted on several occasions that, when they came in contact with the body of a snail, they became more quiescent, and moved about over its surface by means of the sucker for a time, after which they apparently resumed their swimming.

The cercariae escaped from the snail, one at a time, and, in a case specially observed, at intervals of about ten minutes. They then moved towards the surface of the water or towards the light. After swimming about actively for about 24 hours, they were observed to be moving away from the light, and downwards. They then began to crawl, often with a looping movement, along the bottom of the dish, never on the vegetation in the water. Finally they appeared to be endeavouring to attach themselves to the dish, the head meanwhile being bent downwards and the tail moving rapidly. Though closely watched in this attitude they were never observed to become encysted, but, after a time lost their tails, became swollen and died. No encysted forms were found on the walls of the vessel in which the water was contained nor on vegetable matter or mud from the lake from which the snails were procured. It was however noted as a peculiar fact that, of the several hundreds in the dish, not even the dead remains were found, except in a few cases. To check this observation more carefully a dozen cercariae were then removed from the dish containing the snails and put in a test tube, and all of them after a time sank to the bottom, became swollen and died. The solution of this difficulty, and, at the same time, the key to the whole life history, became evident on the finding of an encysted form in the body of the snail itself; this suggested that the cercaria might, after a free life, again return to the snail, and become encysted in it. Further search showed that, in nearly all cases at a certain time, cysts could be found in the snail in a patch of 20 to 200 in the pericardium, clustered round the auricle.

It was difficult to discover exactly how the cercariae entered the body of the snail, as this was effected apparently within the mantle cavity. It was observed several times that cercariae on touching the surface of the body of the snail immediately ceased the wriggling movement of the tail and began to crawl over the surface, in and out of the mantle cavity. One observation however was made which indicated that the cercariae gain access to the pericardium, not by boring through the skin, but by the nephridial opening. In this particular case the snail under observation had the mantle cavity widely open, and the gill protruding; about twenty cercariae were crawling apparently indiscriminately on the surface of the foot, the body and in and out of the mantle cavity. When the animal was placed under the microscope, the nephridial opening was observed near the base of the gill at its anterior right side. The nephridial aperture in some molluscs can best be found by observing the living animal in this way, when it is seen to open and close at intervals—in this case at intervals of 18 seconds. The cercariae, in their active looping movements, passed rather quickly over all the exposed surface, and several of them passed over the nephridial aperture, others even turned away when it opened in front of them. So persistent was this active movement that it seemed as if it had a definite purpose, perhaps procuring food. One was observed however to crawl rapidly into the opening, and soon disappeared into it. The aperture had been watched for about half an hour, and, after several hours, on re-examination, the snail, which had been kept in a watch glass was found to have only two cercariae crawling over it. There can be little doubt that this observation, together with the fact that the cysts are lodged in the pericardium, indicates that the cercariae enter the snail by the nephridial aperture.

A cercaria, crawling about on the surface of the snail, showed indication of the beginning of a change. The projecting shoulders were occasionally well marked, and behind them, on the neck a number of small hooks or bristles were seen.

On another occasion, in March, some snails were procured, in which were found a large number of cysts (over 500), and near them were some very active cercariae not encysted. Some of them possessed tails and some were without. Some possessed two sets of spines, and were apparently about to encyst.

There seems therefore to be no doubt but that the cercariae in question are represented by the cysts. Other means were however taken to confirm this. A number of free cercariae were put with a snail in a test-tube, and a similar number in another test-tube without a snail. In about an hour the first had disappeared, while the dead remains of the second lot were found at the bottom of the tube.

This does not altogether exclude the possibility that the cyst may have arisen from cercariae which had never been outside the snail; this possibility however was disposed of by the finding of a snail with a perfectly healthy liver, but badly infected with cysts.

Such cysts were found in aestivating snails in the mud.

The cysts were 0·156 mm. in diameter and transparent. Each could be observed to contain a small *Distoma* either at rest or revolving slowly in its capsule, and well characterised by an arc of spines in a double row situated along the anterior dorsal edge, and reflected over on each side ventrally for a short distance, where it consisted of two or three spines only; minute spines also appeared on the dorsal surface of the body. Two rows of clear oily looking cystogenous cells were observed in all. Such spines were never observed in the free swimming cercariae.

### FLUKE OR ADULT STAGE.

The next stage in the life history of the encysted cercariae was probably to be found in some animal which lived on the snails, and, as the lake abounded with frogs (*Rana* and *Xenopus*) these were naturally suspected to act as the next host for the parasite. A number of these frogs were dissected, and it was ascertained that both kinds fed extensively on the snails. Among the many kinds of parasites (protozoa, round and flat worms) found in the alimentary tract of the frog were a number of flukes, which were very abundant, differing however from the encysted form in that they had no strong anterior spines, but a great number of small spines arranged in closely set rows (about 40 to 60) on the dorsal and extending to the ventro-lateral sides of the body. The largest of these flukes were about 1·2 mm. in length and 0·32 mm. in breadth. They were of a yellowish colour and some had well developed ova. The species is nearly related to *Distoma echinatum*, but, as the intermediate stage differs somewhat from that of the latter, which moreover is never found in a cold blooded animal, we must regard this as probably a new species, which may be called *D. luteum*.

In order to trace the various stages, and to ascertain if these were the adults of the encysted form a number of feeding experiments were carried out. Some frogs, after being kept without food for a time, were given some of the snails in which cysts, as well as rediae and cercariae, occurred. A frog, dissected shortly afterwards, still had the snail in its stomach; the cysts were unchanged though the rediae and cercariae were digested. Another frog, examined about six hours afterwards, had the remains of the snail with the group of cysts in the small intestine, about half way to the rectum, and, in some cases, the fluke was found in the act of escaping from the cyst, sometimes head first and sometimes tail first, while others had apparently been free for some time and were moving about actively. These were of particular interest as they possessed the large oral spines of the encysted forms, and the small dorsal spines of the fluke above mentioned. This experiment was repeated several times with intervals of three to seventy-two hours between times of feeding and dissection, and examination of all these confirmed the first experiment, though in the last case the empty cysts and well developed flukes only were observed. The confirmation of this by repeated experiments was necessary to prevent confusion with other cercariae and flukes, for the mollusc *Tomichia,* which harbours a cercaria as already mentioned, was occasionally found in the frogs, and there is another fluke, of a larger size and obviously different from that under observation, which is frequently found in the mouth of the frog just behind the tongue and still another occurs in the lungs. The life histories of the cercaria, of *Tomichia* and these flukes are other interesting subjects for investigation.

*The Egg Stage.* The eggs were readily observed in the semi-transparent body of the adult fluke, and were frequently seen being discharged from the body; they were found also in the débris in the rectum. They were oval and varied in size from 0·34 to 0·25$\mu$ in length and from 0·18 to 0·16$\mu$ in breadth and were of a yellowish colour.

The eggs were kept under varying conditions for some considerable time, but no sign of the emergence of an embryo was observed. It may be that no free swimming larva is developed and the eggs are swallowed by the snail.

# A STUDY OF THE DIVERSE RACES OF *ENTAMOEBA HISTOLYTICA* DISTINGUISHABLE FROM ONE ANOTHER BY THE DIMENSIONS OF THEIR CYSTS.

By CLIFFORD DOBELL
(*Imperial College of Science*)
AND MARGARET W. JEPPS
(*Bathurst Student, Newnham College, Cambridge*).

(With 7 Text-figures and Plate VI.)

(*A REPORT TO THE MEDICAL RESEARCH COMMITTEE*[1].)

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## INTRODUCTION.

In a recent paper (1917) we have drawn attention to the fact that there is great variation in the size of the cysts formed by the dysentery amoeba,

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[1] The work here described was chiefly carried out, with the aid of grants from the Medical Research Committee, at the Wellcome Bureau of Scientific Research. We wish to record our indebtedness once more to the Bureau, for its unfailing help, and for its hospitality whilst the work was in progress.

*Entamoeba histolytica*[1]. We stated that there are different races or strains of this species distinguishable from one another by the dimensions of their cysts, our conclusions in this respect agreeing closely with those of Wenyon and O'Connor (1917). In the following pages we propose to present in greater detail the evidence for our conclusions. Before doing so, however, it is necessary to enter into some detail concerning certain preliminary investigations which were indispensable for a proper demonstration of this fact. Therefore, we shall first consider these necessary preliminaries; then present our evidence and the conclusions which we have drawn from it; and finally discuss the results of some other observers in the light of our own findings.

## METHODS AND RESULTS.

### Part I.

### A. *Methods used to determine the dimensions of the cysts of* E. histolytica.

An exact determination of the dimensions of the cysts of *E. histolytica* is by no means as easy as it might at first sight appear. The cysts are of small size, seldom perfectly symmetrical, and subject to considerable modification in various respects by the medium in which they are measured. It is moreover necessary to make a large number of measurements before any definite conclusion can be drawn from them. We therefore think it important to give in detail the methods we have adopted for this investigation.

When the cysts are carefully examined under the microscope it is found that very few indeed are perfectly spherical[2]. Even these which at first sight appear to be so, will usually be found not truly spherical if moved and viewed from other aspects. If we were to confine our attention therefore to those cysts which are perfectly spherical, and thus capable of exact and rapid measurement, we should ignore the vast majority of cysts in any infection; a procedure which might lead to a totally false estimate of the dimensions of the cyst population as a whole. Accordingly we have ignored only those cysts which were much misshapen and which formed only a very small proportion of the total number in the infections here described; and we have measured all those which were approximately elliptical or circular in outline. The size of the latter was recorded simply as the diameter, and of the former as the mean of the longer and shorter axes.

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[1] We continue to use this name, rather than *E. dysenteriae*, in referring to this organism, for reasons given elsewhere by one of us (Dobell, 1918).

[2] Cf. the outlines of cysts shown in the figures on Plate VI. We have seen it stated several times that the living cysts of *E. histolytica* are in reality perfectly spherical, and that fixed and stained cysts of different shape have been produced artificially in making the preparations. This may be the rule when the technique employed has been bad; but we find it hard to believe that anybody who has carefully studied a large number of cysts, both living and stained, and in many different infections, can uphold such an opinion. Most living cysts are not perfectly symmetrical; and obviously asymmetrical living cysts are found commonly in most infections and in some almost exclusively. Although, as will be shown in experiments recorded below, fixation—even with a good fixative—may alter the shape of a cyst, it does not appreciably modify the majority: and we shall show that, when fixation does change the shape of a cyst, this change may actually be from an oval to a spherical shape.

All the measurements were made with a finely-ruled ocular micrometer, in combination with a 1/12″ oil immersion objective. The microscope was adjusted in all cases so that one division of the micrometer scale represented 1·5$\mu$. We always estimated the diameter of a cyst to the nearest half of a division, so that the unit employed was in reality 0·75$\mu$. All the measurements were recorded directly in units of the micrometer scale, and not converted into microns until afterwards. Perfectly accurate measurements cannot of course be obtained by this method, but we know of none better for obtaining a large series of fairly trustworthy measurements within a reasonable time.

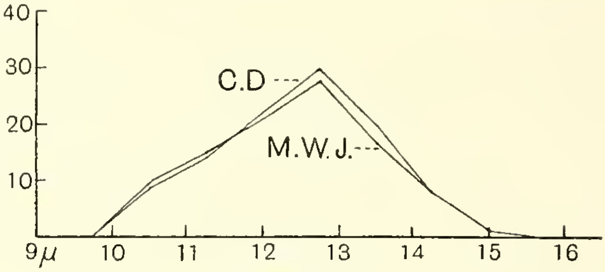

Fig. 1.

Since every measurement made in this manner involves a certain amount of estimation on the part of the measurer, it is clear that the results recorded by different individuals are likely to differ slightly from one another. We endeavoured to determine our own individual differences in this respect in the following way. One hundred cysts in a fixed and stained preparation were measured as carefully as possible, each cyst being measured by each of us independently, and the measurements independently recorded. On comparing the results we found that the dimensions registered were identical for 71 of the cysts. For each of the remaining 29 cysts there was a difference of half a micrometer division, or 0·75$\mu$, between the two measurements. One of us (M. W. J.) recorded the diameters of 18 of these 29 cysts as half a division smaller, and 11 as half a division larger, than the other (C. D.). The two series of measurements when recorded graphically gave curves of closely similar form (Fig. 1). We concluded therefore that for the majority of cysts measured our findings would be identical, whilst in the case of the remainder the individual error would not be greater than one unit of the scale employed: and furthermore that this error would probably not influence the character of our curves. We decided to measure a large number of cysts (500) in every infection studied, in order to obtain an approximately true average value for the sizes of the cysts; and to measure half (250) of this number each in every 500, so as to eliminate as far as possible the personal error. Each series of measurements here recorded was obtained in this way.

As we recorded all our measurements in units of the micrometer scale we found it most convenient to plot our curves in these terms. The conversion of the units into microns, as shown in the charts, was made directly on the charts themselves. For this reason the points on the curves do not always coincide with the integral values in microns on the abscissae.

### B. *Determination of the differences in the dimensions of cysts when examined alive and after fixing, staining, and mounting: with an inquiry into the factors which determine these differences.*

It is well known that the dimensions of cysts after fixation, staining, and mounting are not the same as those of the living cysts. As, however, it was not always possible to measure large numbers of living cysts at the time when they were obtained, it became necessary to rely upon fixed and stained material; and it therefore became necessary also to ascertain what effect this would have on our measurements. To determine this point we adopted two different methods: first, the measurement of a large number of cysts (from the same infection) both living and when fixed, stained, and mounted—the mean dimensions so obtained being then compared; and secondly, we devised a method for observing and measuring an individual cyst during the various processes of fixation, staining, and mounting in balsam. Throughout this paper the expression "fixed, stained and mounted in balsam" signifies—unless the contrary is stated—that fixation was effected with a modified form of Schaudinn's fluid (saturated corrosive sublimate in water, two parts; absolute alcohol, one part; glacial acetic acid, 4–5 %), and staining with Mayer's haemalum; and that the mounting medium was Canada balsam dissolved in xylol. This procedure was adopted because it was that which, after a long series of trials of various methods[1] made by one of us (C. D.), was found to give the most reliable, uniform, and rapid results in practice. We have long used this, therefore, as a routine method. Cysts fixed in this manner are not as a rule appreciably shrunken or distorted; and although the stain is inferior in some respects to iron-haematoxylin and some other methods, it permits of the study of considerable cytological detail, whilst demanding but a small fraction of the time, labour, and attention requisite for these.

The cysts selected for the first method of investigation were obtained from a convalescent dysentery patient (E. 42), who had long been under observation, and whose cysts were of a conveniently large size. From a stool passed by this patient we measured

(1) 500 living cysts in 0·75 % saline solution;
(2) 500 cysts in Weigert's iodine solution;
(3) 500 cysts fixed, stained, and mounted in Canada balsam;
(4) 500 cysts similarly fixed and stained, but mounted in Gilson's euparal.

Our results are given in the following Table (Table I) and recorded graphically in Fig. 2.

TABLE I.

| | Dimensions of cysts in units of ocular micrometer*. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5·5 | 6·0 | 6·5 | 7·0 | 7·5 | 8·0 | 8·5 | 9·0 | 9·5 | 10·0 | 10·5 | 11·0 |
| No. of cysts in saline | — | — | — | 7 | 28 | 130 | 108 | 136 | 52 | 32 | 3 | 4 |
| ,, ,, iodine | — | — | — | 16 | 29 | 117 | 126 | 134 | 45 | 26 | 7 | — |
| ,, ,, balsam | 1 | 0 | 9 | 60 | 90 | 165 | 101 | 54 | 12 | 8 | — | — |
| ,, ,, euparal | — | 1 | 3 | 49 | 86 | 166 | 101 | 67 | 21 | 5 | 1 | — |

* As explained above, 1 micrometer division = 1·5$\mu$.

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[1] In the course of this work it was found that the majority of the ordinary cytological fixatives give inconstant results with *E. histolytica* cysts, though many are at times excellent. Schaudinn's fluid, for example (without acetic acid), frequently gives results quite as good as those obtained with the liquid mentioned above. Its action, however, is less uniform. It appears immaterial whether 4 % or 5 % of glacial acetic be added, but the addition of either more or less gave less satisfactory results. The only other fixative which gave equally uniform and excellent fixation was a mixture consisting of four parts of saturated picric acid in 90 % alcohol and one part of glacial acetic acid. The only objection to the use of this fluid for routine work is the length of time necessary to wash out the picric acid before staining.

The mean sizes of the cysts calculated from these figures and expressed in microns are as follows:

Mean size of cysts in saline = 12·99 $\mu$ or approximately 13 $\mu$
,, ,, iodine = 12·86 $\mu$ ,, ,, 12·9 $\mu$
,, ,, balsam = 12·04 $\mu$ ,, ,, 12 $\mu$
,, ,, euparal = 12·19 $\mu$ ,, ,, 12·2 $\mu$.

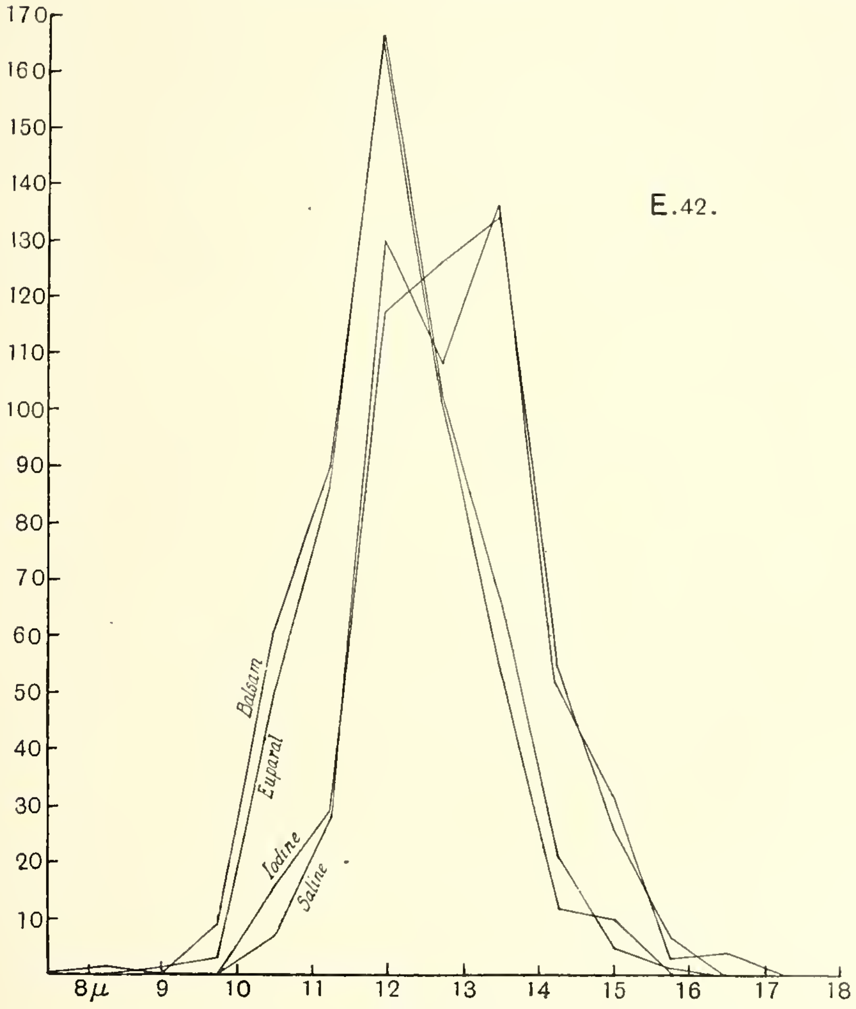

Fig. 2.

The differences in the dimensions of the cysts measured in different media are very clearly shown by the curves[1] (Fig. 2). It will be noted that the curves representing the measurements of the cysts in euparal and balsam coincide very nearly, each having a single apex at 12 $\mu$. The curves for the cysts in saline and iodine are also to a great extent superimposed; but whilst the latter possesses a single summit at 13·5μ, the former displays two apices situated at 12μ and 13·5μ, although numerous cysts of intermediate size are also recorded.

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[1] In these curves, and in all the others in this paper, the numbers of cysts measured are shown on the ordinates, and the dimensions of the cysts in microns along the abscissae.

The curve obtained from the measurements in saline was so curiously different in character—having two apices instead of one—that we were at a loss to account for it. It occurred to us that a possible explanation might be that, in measuring the cysts, we had unconsciously tended to estimate the dimensions to the nearest line on the micrometer scale (12μ and 13·5μ represent respectively eight and nine divisions of the scale): and that we might thus have distributed numerous cysts of intermediate size to those categories immediately above and below them. We decided therefore to make another series of measurements from the same case. Accordingly, a month later, we obtained another sample of faeces from this patient, and measured another 500 cysts in saline. The results were as follows:

TABLE II.

| | Dimensions of cysts in units of ocular micrometer. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 7·0 | 7·5 | 8·0 | 8·5 | 9·0 | 9·5 | 10·0 | 10·5 | 11·0 |
| No. of cysts | 7 | 15 | 90 | 133 | 121 | 74 | 45 | 10 | 5 |

These figures, when presented graphically (Fig. 3), give a curve with a single summit like the others—the summit in this case being at 12·75μ or midway between the two summits of the first saline curve (Fig. 2). As we have never at any other time obtained a curve of the latter type from a series of measurements of *E. histolytica* cysts, we believe that in this case it was an accidental result of factors which we cannot at present specify with precision, though we believe the explanation suggested above is probably correct.

On comparing the average dimensions of the cysts measured in different media, it will be seen that the size of those measured in saline and iodine is approximately the same (12·99μ and 12·86μ), whilst that of those measured in balsam is less (12·04μ). We concluded therefore that the action of iodine has no effect upon the size of the cysts—neither shrinking them nor causing them to swell—when used as a fixative: but that the apparent size after fixing, staining, and mounting in balsam is appreciably diminished. This diminution in average size amounted in the present case to approximately 1μ.

To what is this apparent shrinkage due? A careful study of the cysts in saline and balsam seemed to us to indicate quite clearly that it is due merely to the circumstance that the cyst wall is clearly visible in the former medium, but almost always invisible in the latter. The diameter of a cyst measured in saline therefore includes twice the thickness of the cyst wall, whereas that of a cyst mounted in balsam is really the diameter of the stained protoplasmic contents only. This supposition is supported by the measurements made of cysts in euparal. As is well known, the refractive index of euparal is lower than that of Canada balsam dissolved in xylol; and accordingly the walls of cysts mounted in the former medium are frequently visible, and would therefore be often included in measurements of their diameters. We should therefore expect that the mean size of cysts mounted in euparal would be intermediate between the sizes of those in saline and those in balsam. This we found to be the case, the mean size of cysts in euparal being 12·2 $\mu$. If this explanation is correct we should expect further that the cysts actually measured in balsam would, if remounted in a medium of lower refractive index, be found to have a greater mean size. To test this we remounted in water the coverglasses which had been originally mounted in balsam, and from which the above measurements had been made. Five hundred cysts were again measured in them, and the mean size was found to be 12·3 $\mu$ as against 12·04 $\mu$ for the same cysts in balsam. This increase in size, though appreciable, was not so great as we had expected: but we considered that the measurements of the cysts in water were not altogether trustworthy. It was often extremely difficult, if not impossible, to distinguish

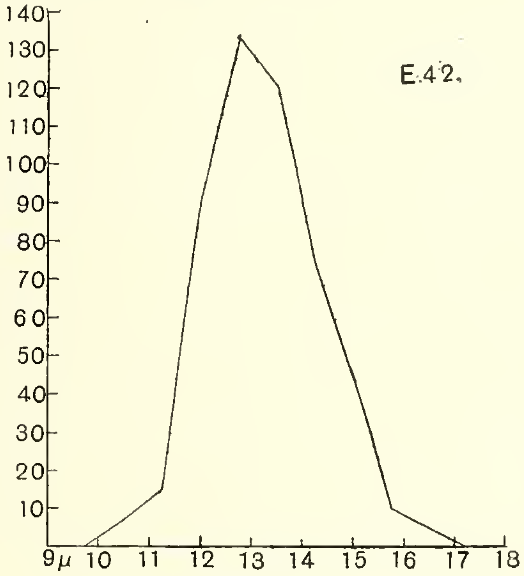

Fig. 3.

the outline of the cyst wall sharply, on account of the opacity of the stained cysts and the surrounding bodies in the preparation.

More direct evidence was obtained by measurements of the thickness of the wall of individual cysts. The cyst wall of *E. histolytica* is extremely thin, and consequently difficult to measure. We were able, however, to obtain some satisfactory measurements by means of the camera lucida. The cysts were studied under a 2 mm. apochromat (N.A. = 1·40), with compensating ocular 18, and with critical illumination from an achromatic aplanatic condenser (N.A. = 1·40), the measurements being read off directly from the projected image at a magnification of 2500 diameters. The thickness of the cyst wall was measured in living cysts, in cysts plasmolysed in 10 % sodium chloride solution, and in fixed, unstained cysts suspended in 70 % alcohol. The thickness of the cyst wall was found to be remarkably uniform. In no case, after making between 30 and 40 measurements, was it found to be less than 0·5$\mu$ or greater than 0·6$\mu$. The mean value for 20 such measurements was 0·506$\mu$. It should be added that the cysts used for these measurements were of approximately the same size as those from case E. 42, but were obtained from two different patients.

These figures are in complete agreement therefore with the supposition made above, namely, that the decrease in size observable in cysts mounted in balsam is due chiefly to the invisibility of the cyst walls in this medium. If the thickness of the cyst wall is 0·5–0·6$\mu$, we should expect the diameters of cysts in saline or iodine—in which the cyst walls are visible—to be on an average greater by twice this amount (1$\mu$–1·2$\mu$) than the diameter of those in balsam—in which the cyst walls are not visible. And, as we have already stated, the mean diameter of cysts in balsam was actually found to be approximately 1$\mu$ less than that of cysts measured in saline or iodine solution.

It is clear that a direct proof of the correctness of this interpretation could be obtained if individual cysts could be studied microscopically both when alive, and during all the processes of fixation, staining, and mounting. As noted above, we succeeded, after some failures, in devising a simple method of doing so. Our procedure is as follows. A small sample of faeces containing cysts of *E. histolytica* is mixed on a slide with a suitable quantity of gelatine jelly previously liquefied by warming. Before the gelatine has time to set, a small drop of this emulsion is spread in the middle of a coverglass (7/8″ × 7/8″), and the latter placed inverted in the concavity of a hollowground glass block (6 cm. × 6 cm., 1·25 cm. thick, with a deep concavity 5 cm. in diameter). The coverglass thus stands on its four corners in the middle of the concavity, with its film downwards. The corners are then firmly cemented to the block with drops of paraffin wax. The preparation is then placed on the stage of the microscope and the film examined under a low power. When a cyst of *E. histolytica* has been found, it is carefully placed under the oil immersion in the centre of the field, and measured with the ocular micrometer. When the gelatine has set, the fixative is introduced with a fine pipette under the coverglass, and allowed to fill completely the space between it and the block. When this operation is properly performed, the cyst, which is observed continuously during the process, will be found to remain in position and not to be dislodged by the entry of the fixative. Its measurements after fixation may then be again recorded.

In carrying out the above process, the following points should be kept in mind, since the success of the experiment depends upon them. The gelatine solution used must be of such concentration that it will liquefy at a very low temperature (so as not to injure the cysts) but set at the ordinary temperature of the laboratory. The concentration of salts in it must also be such that living cysts are unaffected by it. We found a 5 % solution of gelatine in physiological saline solution (0·75 % sodium chloride in distilled water) to fulfil these requirements perfectly. Cysts in this medium appear perfectly normal under the microscope, and remain, in both the sol and the gel, unstainable with eosin (Kuenen and Swellengrebel's test of the vitality of cysts). We tried a jelly deeply coloured with eosin in the expectation that the cysts would be more sharply defined, and therefore easier to measure, in this medium. We found, however, that the colourless jelly is preferable. The film must be made of a suitable thickness, which can only be determined by a few trials. It must not be so thick as to prevent exact measurement of the cyst after fixation of the jelly, nor so thin that the gelatine rapidly undergoes desiccation after it is set. The fixative must be introduced when the gelatine has completely set, but before it has had time to dry. The setting of the gelatine can easily be determined under the microscope by observing the complete cessation of Brownian movement in the smallest particles in the preparation.

We made a number of observations by this method, and give below the results in tabular form (see Table III). The fixative used in all these experiments was that described above (sublimate-alcohol-acetic-acid). In the table the first measurement given for each cyst is the vertical diameter, the second the horizontal. Fixation of the cysts as seen under the microscope was almost instantaneous. As a rule the measurements after fixation were made within five minutes of the application of the fixative, but in some cases they were repeated at intervals up to one and a half hours later. When this was done we found the prolonged action of the fixative produced no subsequent change in size. The measurements in every case were made by one of us and checked by the other before being recorded. The cysts were from an infection in which they were of large size, and thus convenient for measuring.

TABLE III.

| | Dimensions of cyst in microns | | |
|---|---|---|---|
| Cyst No. | Before fixation | After fixation | Effect of fixation on size of cyst |
| 1 | 13·5 × 15 | 13·5 × 15 | None |
| 2 | 13·5 × 12·75 | 12·75 × 13·5 | None (slight change of shape) |
| 3 | 14·25 × 14·25 | 14·25 × 14·25 | None |
| 4 | 13·5 × 13·5 | 13·5 × 13·5 | None |
| 5 | 13·5 × 15 | 13·5 × 15 | None |
| 6 | 14·25 × 13·5 | 14·25 × 13·5 | None |
| 7 | 12·75 × 12 | 12·75 × 12 | None |
| 8 | 15 × 14·25 | 15 × 14·25 | None (slightly distorted) |
| 9 | 15·75 × 14·25 | 15 × 15 | None (slight change of shape) |
| 10 | 14·25 × 13·5 | 14·25 × 13·5 | None |
| 11 | 15 × 14·25 | 15 × 14·25 | None (slightly blistered) |
| 12 | 13·5 × 13·5 | 13·5 × 13·5 | None (slightly blistered) |
| 13 | 14·25 × 12·75 | 15 × 12 | None (change of shape) |
| 14 | 16 × 14·5 | 16 × 14·5 | None. |

From these experiments we conclude that living cysts, when fixed with the solution which we have used, undergo no change in size whatever as the result of fixation.

The effects of staining, dehydration, clearing, and mounting in balsam were studied under the microscope in the same way as the effects of fixation: that is to say, the fixative was removed with the pipette, and other reagents substituted successively as required. We give below the results of three such experiments in detail.

*Expt.* 1. (Cyst No. 4 of Table III).

| Medium in which cyst was examined | Dimensions |
|---|---|
| Gelatine jelly (living cyst) ... ... | $13{\cdot}5\mu \times 13{\cdot}5\mu$ |
| Fixative (after 5 mins.) ... ... | $13{\cdot}5\mu \times 13{\cdot}5\mu$ |
| Fixative (after 30 mins.) ... ... | $13{\cdot}5\mu \times 13{\cdot}5\mu$ |
| 70 % alcohol ... ... ... ... | $13{\cdot}5\mu \times 13{\cdot}5\mu$ |
| Paracarmine (20 mins.) ... ... | |
| Absolute alcohol ... ... ... | $13{\cdot}5\mu \times 13{\cdot}5\mu$ |
| Xylol ... ... ... ... ... | $12{\cdot}75\mu \times 12{\cdot}75\mu$ |
| Balsam (immediately) ... ... | $12\mu \times 12\mu$ |
| Balsam (after $1\frac{1}{2}$ hrs.) ... ... | $12\mu \times 12\mu$ |

It will be seen that in this experiment the size of the cyst was unchanged by fixation, staining, and dehydration; but that a slight diminution in size was observed in xylol, and a further small reduction in balsam. This was clearly due to the circumstance that the cyst wall was still partly visible in the former, but completely invisible in the latter. The cyst wall remained unstained by the paracarmine; and there could be no doubt that the measurements of the "cyst" in balsam were really those of the stained protoplasmic contents only.

*Expt.* 2. (Cyst No. 7 of Table III).

| Medium in which cyst was examined | Dimensions |
|---|---|
| Gelatine jelly (living cyst) ... ... | $12{\cdot}75\mu \times 12\mu$ |
| Fixative (after 5 mins.) ... ... | $12{\cdot}75\mu \times 12\mu$ |
| Fixative (after 1½ hrs.) ... ... | $12{\cdot}75\mu \times 12\mu$ |
| 50 % alcohol ... ... ... ... | $12{\cdot}75\mu \times 12\mu$ |
| Paracarmine (20 mins.) ... ... | |
| Absolute alcohol ... ... ... | $12{\cdot}75\mu \times 12\mu$ |
| Xylol ... ... ... ... ... | |
| Balsam (immediately) ... ... | $10{\cdot}5\mu \times 9\mu$ |
| Balsam (after 1½ hours) ... ... | $10{\cdot}5\mu \times 9\mu$ |

Continuous observation of the cyst showed that the final decrease in size took place as follows. In absolute alcohol the protoplasm underwent a very slight shrinkage from the cyst wall. (The measurements in this medium are those of the cyst wall—not of the contents). In xylol considerable further contraction of the protoplasm was observed, a space separating the contents from the wall. In balsam the cyst wall, and consequently the space between it and the protoplasm, became completely invisible. The greatly reduced dimensions are therefore those of the shrunken protoplasmic contents only.

*Expt.* 3. (Cyst No. 13 of Table III).

In the two previous experiments the staining of the cyst was effected with paracarmine (in 70 % alcohol) in order to avoid the tedium of transferring the cyst through the many grades of alcohol necessary in using a watery stain. We considered it desirable, however, to make further observations on cysts stained in haemalum, since this is the method we have employed as a routine throughout our investigations. We soon found, however, that the cysts were liable to drop out of the film when transferred to water, unless the gelatine was thoroughly hardened after fixation, and numerous grades of alcohol employed. In the following experiment, the gelatine was hardened in formol-alcohol (formol (40 % formaldehyde) and absolute alcohol, equal parts). As will be seen this procedure had no visible effect on the dimensions of the cyst. Measurements were made in each grade of alcohol used.

| Medium in which cyst was examined | Dimensions |
|---|---|
| Gelatine jelly (living cyst) | 14·25μ × 12·75μ |
| Fixative (after 5 mins.) | 15μ × 12μ |
| Fixative (after 40 mins.) | 15μ × 12μ |
| Formol-alcohol (after 2½ hrs.) | 15μ × 12μ |
| Alcohols 35 %, 20 %, 10 % | 15μ × 12μ |
| Distilled water | 15μ × 12μ |
| Haemalum (15 mins.) | |
| Tap water | 15μ × 12μ |
| Alcohols 10 %, 20 %, 35 %, 50 %, 70 %, 90 % | 15μ × 12μ |
| Absolute alcohol | 15μ × 12μ |
| Xylol | 13·5μ × 10·5μ |
| Balsam (after 20 mins.) | 13·5μ × 10·5μ |

It will be apparent from the foregoing figures that the dimensions of the cyst underwent no change until its final transference to xylol and balsam. (There was a slight change in shape, but not in size, in the fixative.) Here again the final reduction in size was clearly seen to be due to the disappearance of the cyst wall in the clearing and mounting media.

It will be noticed that in Expts. 1 and 3 the actual loss in diameter observed was 1·5μ, whilst in Expt. 2 there was a loss of 2·25μ in the longer, and of 3μ in the shorter diameter of the cyst. If the diminution in size had been due only to the disappearance of the cyst wall, there should have been only a loss of 1μ–1·2μ in the diameters. In Expt. 2 the greater reduction was clearly due to the shrinkage of the protoplasmic contents; and it seems reasonable to suppose that the slightly greater loss than we expected in the other two experiments was due to the same cause. It will be remembered that in our earlier observations on the mean size of 500 cysts in saline solution and in balsam, we actually found a difference in average diameter of only about 1μ. We think that this was probably due to the fact that the fixed cysts were very thoroughly hardened in alcohol before staining and mounting, in order to prevent shrinkage as far as possible: and, in measuring these cysts subsequently, we rejected all those which showed signs of shrinkage.

In all the experiments hitherto described, we have confined our attention to cysts of large size (with diameters usually of about 12–14 μ). Our conclusions, therefore, are directly applicable to cysts of these dimensions only. The difficulty of manipulating very small cysts, and the much greater difficulty of discounting errors in the measurement of objects of very small dimensions, deterred us from attempting to extend our observations to cysts of smaller size. It seemed to us almost certain, however, that closely comparable results would have been obtained for these. We have assumed, therefore, when dealing with the smaller-sized cysts of *E. histolytica*, that the effects of mounting in balsam would be the same, and the apparent reduction in size proportionate.

The foregoing experiments supply, we think, conclusive solutions of the problems which we set ourselves. We may therefore now summarize our results up to this point.

1. Cysts of *E. histolytica* which have been suitably fixed and stained and mounted in balsam, appear to possess a smaller mean diameter than those examined alive in saline solution, or after treatment with iodine. (There appears, however, to be no appreciable difference between the size of living cysts suspended in saline and those killed and suspended in Weigert's iodine solution.)

2. When properly fixed the cysts do not undergo any appreciable shrinkage in the fixative or in the usual reagents employed in staining and dehydration. The apparent loss of size takes place in the process of clearing and mounting; and is chiefly due to the disappearance of the cyst wall when the cyst is placed in a medium of high refractive index. Shrinkage of the protoplasmic contents from the cyst wall may also, in some cases, account for a still further diminution in the apparent diameter.

3. The average thickness of the cyst wall of *E. histolytica* was found to be 0·5 μ. In an ideal case, therefore, in which no protoplasmic shrinkage occurs, and the apparent diminution of the diameter of the mounted as compared with the living cyst is due only to the disappearance of the cyst wall in the mounting medium, the difference in diameter should be approximately 1 μ. Comparative measurements of living cysts and of those mounted in balsam with due precautions to prevent shrinkage and discount its effects, actually gave a difference of this magnitude in the mean diameters of the cysts. Shrinkage of the protoplasm may, however, lead to a considerable further reduction in size. (In one case directly observed under the microscope there was a reduction in the mean diameter of 2·6 μ.)

4. Assuming that the figures given above—which were obtained from a study of large cysts—are proportionately applicable to cysts of all sizes, we conclude as follows: When the processes of fixing, staining, and mounting are suitably conducted, so that protoplasmic contraction is avoided or reduced to a minimum, there is an apparent reduction of about 10 % in the diameter of a cyst mounted in balsam as compared with its true diameter in the living condition.

## Part II.

### *Demonstration of the existence of races of* E. histolytica *which differ from one another in the size of the cysts which they produce.*

In our earlier paper (1917) we briefly noted some results obtained in the study of cysts from over 200 patients infected with *E. histolytica*. We pointed out that there is a very wide range of variation in the size of the cysts passed by different persons; the extreme limits which we observed in this respect being represented by diameters of $5\mu$ and $20\mu$ for spherical cysts, examined alive in saline solution or killed and suspended in iodine. The measurements which we are now about to give were all made upon cysts which had been previously fixed, stained, and mounted in balsam. In comparing them, therefore, with our former figures it will be necessary to introduce the correction indicated in the preceding section of the present paper. The cases here described are, however, for the most part selected from the same series. So far as our technique is concerned, it will suffice to note that it has been uniform throughout, and is that described above. We must emphasize once more, however, that we have endeavoured as far as possible to eliminate the error due to protoplasmic shrinkage, by thorough hardening of the cysts before mounting them, and by omitting distorted or obviously shrunken cysts from our measurements. We believe, therefore, that the difference in the size of the cysts due to cytological manipulation approximates fairly closely to the ideal value to be expected from our preliminary investigations. Furthermore, as already noted, our conclusions are based upon measurements of 500 cysts in every case—250 measured by each of us independently.

Measurements of the cysts from one case (E. 42) in our series have already been given in some detail on an earlier page (see Table I). The cysts of this strain of *E. histolytica* are of comparatively large size, the summit of the curve plotted from them being at $12\mu$ for the fixed and stained cysts. Strains such as this are, in our experience, the commonest.

We find, however, that there are strains which produce cysts having a mean size either smaller or greater than this. Strains of much smaller size—the commonest being those with an average diameter of 7 $\mu$ to 8 $\mu$—are not uncommon; but strains with a diameter considerably greater are by no means common. We have already (1917) noted that, in a series of over 200 *E. histolytica* infections which we studied, over 30 % were infections with cysts of the smaller sizes. But we have seen so few infections with cysts having a mean diameter greater than 14 $\mu$ that we are at present unable to form any accurate estimate of their real frequency.

In the following table (Table IV) we give the results of four series of measurements of the cysts from four different carriers of *E. histolytica*. The measurements, as before, are all given in units of the micrometer scale—not in microns. Case E. 42 has already been recorded (see Table I, line 3), but is repeated here for comparison with the others.

### TABLE IV.

*Measurements of cysts belonging to four different strains of* E. histolytica.

| | Dimensions of cysts (in balsam) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Case No. | 3·0 | 3·5 | 4·0 | 4·5 | 5·0 | 5·5 | 6·0 | 6·5 | 7·0 | 7·5 |
| H. 8. ... | 8 | 33 | 194 | 140 | 102 | 19 | 3 | 1 | — | — |
| H. 7 ... | 1 | 6 | 44 | 93 | 205 | 105 | 37 | 8 | 1 | — |
| E. 42 ... | — | — | — | — | — | 1 | 0 | 9 | 60 | 90 |
| B. 1 ... | — | — | — | — | — | — | 1 | 1 | 11 | 18 |

| | Dimensions of cysts (in balsam) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Case No. | 8·0 | 8·5 | 9·0 | 9·5 | 10·0 | 10·5 | 11·0 | 11·5 | 12·0 | 12·5 |
| H. 8 ... | — | — | — | — | — | — | — | — | — | — |
| H. 7 ... | — | — | — | — | — | — | — | — | — | — |
| E. 42 ... | 165 | 101 | 54 | 12 | 8 | — | — | — | — | — |
| B. 1 ... | 57 | 92 | 152 | 71 | 55 | 23 | 5 | 7 | 6 | 1 |

This table displays very clearly the differences in the dimensions of the cysts in these four races. The differences are, however, seen in a still more striking manner in the figures[1] reproduced in Plate VI, and in the curves plotted from the measurements. To facilitate comparison we have plotted the four series together in a single chart (Fig. 4). In this, as in the others, the units of measurement have been converted into microns. It will be seen that the summit of the curve for each race falls

[1] A full explanation of these figures is given in the description of the plate.

at a different point: and if we apply the correction indicated in Part I of this paper, we get the following figures for the diameters of the living cysts which occur with greatest frequency in each of these four races:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Case | H. 8, | greatest | frequency | 6·0$\mu$, | representing | 6·6$\mu$ | for living | cyst |
| „ | H. 7 | „ | „ | 7·5$\mu$ | „ | 8·3$\mu$ | „ | „ |
| „ | E. 42 | „ | „ | 12·0$\mu$ | „ | 13·3$\mu$ | „ | „ |
| „ | B. 1 | „ | „ | 13·5$\mu$ | „ | 15·0$\mu$ | „ | „ |

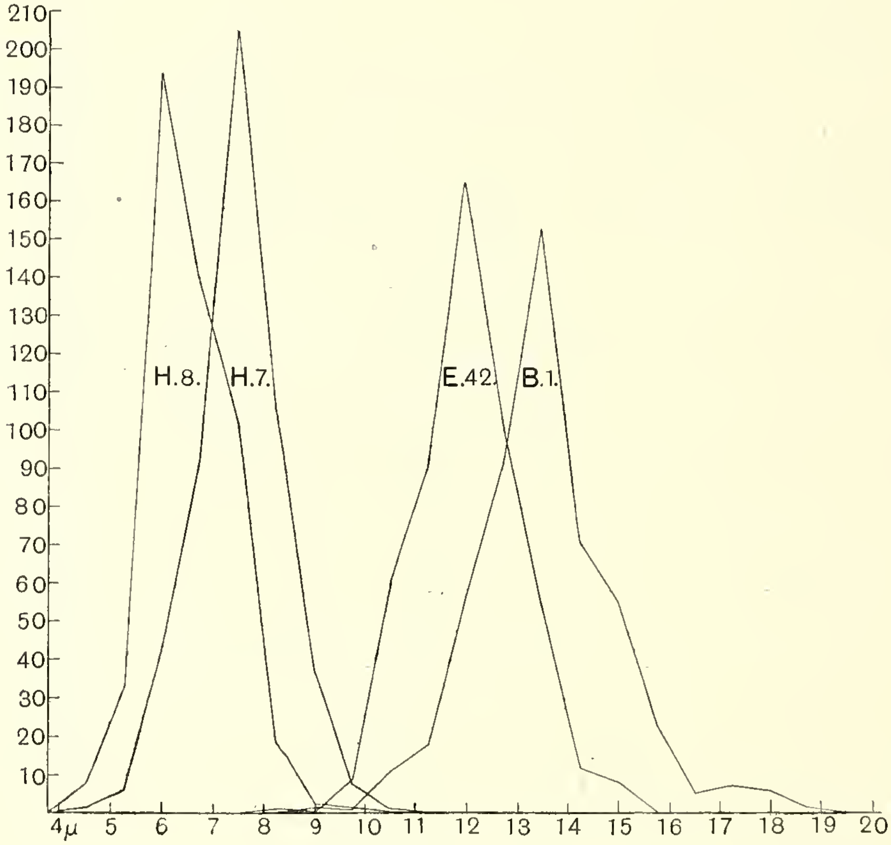

Fig. 4.

The figures calculated in this way for the living cysts were borne out by those which we had previously recorded after measuring a number of cysts in the fresh stools. These measurements were made during the ordinary routine examination of the stools of these patients, and are therefore based upon a comparatively small number of observations. Case H. 8, was recorded as having cysts about 6·5$\mu$ in diameter; Case H. 7, small cysts, variable in size, usually about 8–9$\mu$; Case E. 42, cysts about 12–14$\mu$; whilst Case B. 1 was merely noted as having many large cysts 15$\mu$ or more in diameter[1].

Attention must here be drawn to the character of the curves obtained from each of our series of measurements. Inspection of Figs. 1–4 will convince the reader, we believe, that all the curves are of essentially the same form. They are unimodal, each with a single apex, and, like a normal curve of error, approximately symmetrical about the mode. With the exception of one curve (saline) shown in Fig. 2—which we have already discussed and concluded to be abnormal—we have up to the present obtained no curves which are not conformable to this type. We conclude from these findings that, in each case so far described, we are dealing with a single pure race of *E. histolytica* producing cysts whose average diameter is constant, and which show merely those variations in size from the mean which are to be expected in any biological species or strain displaying the usual degree of variability.

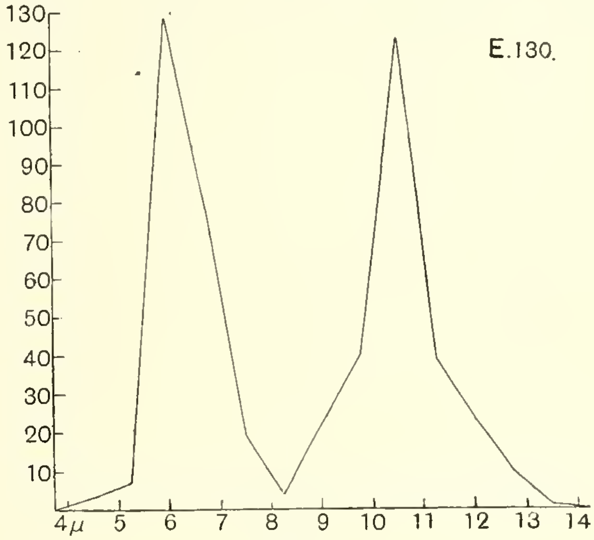

Fig. 5.

For certain other cases, however, a different type of curve was obtained. Three such cases are shown in graphic form in Figs. 5[2], 6 and 7; the actual measurements upon which these curves are based being given in the following table (Table V). The measurements, as before, are given in units of the micrometer scale.

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[1] It is just possible that it may occur to some reader that the large size of many of the cysts in the infections E. 42 and B. 1 is due to the inclusion of cysts of *E. coli* in the series measured. We would therefore state explicitly that neither of these cases is infected with this species, and that cysts containing more than four nuclei have never been found in the stools of either.

[2] The cysts from this case (E. 130) were stained with Weigert's iron-haematoxylin—not with haemalum as were the others.

### Table V.

| | Dimensions of cysts (in balsam) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Case No. | 3·0 | 3·5 | 4·0 | 4·5 | 5·0 | 5·5 | 6·0 | 6·5 | 7·0 | 7·5 | 8·0 | 8·5 | 9·0 |
| E. 130 ... | 3 | 7 | 131 | 78 | 19 | 4 | 22 | 40 | 123 | 39 | 23 | 10 | 1 |
| E. 79 ... | — | 2 | 52 | 59 | 89 | 28 | 24 | 44 | 68 | 63 | 56 | 12 | 3 |
| H. 11 ... | — | — | 7 | 16 | 12 | 3 | 35 | 86 | 160 | 97 | 64 | 18 | 2 |

The outstanding feature of these curves is that they all possess two apices. Case E. 130 (Fig. 5) is perhaps the most striking. The distribution of the cysts around the two modes (6$\mu$ and 10·5$\mu$) is remarkably symmetrical; and the entire curve may be compared with the two separate curves, taken together, from Cases H. 8 and E. 42 (shown in Fig. 4). The latter represented two different strains of *E. histolytica*—from two different patients—and it can hardly be doubted that a similar interpretation must be applied to the former (Case E. 130): for here we obviously have an infection—in this case of the same patient—with two different strains simultaneously, the cysts belonging to each strain being present in approximately equal numbers. The other two cases (E. 79 and H. 11) give less symmetrical curves, but are obviously capable of like interpretation. Case H. 11, for example (see Fig. 7), clearly represents a double infection in which the number of cysts belonging to one strain (that producing large-sized cysts) greatly exceeds that of the other (that producing small cysts).

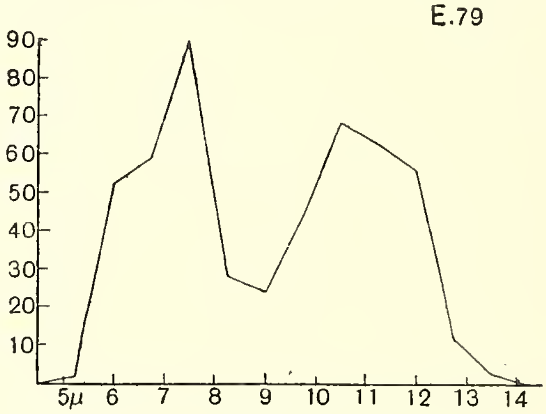

Fig. 6.

The coexistence of two strains of *E. histolytica* in the same patient—such, for example, as is seen in Case E. 130—is sometimes obvious from a mere inspection of a preparation containing the cysts. Nearly all appear to be either large or small, intermediates being either extremely scarce or apparently absent. In our earlier paper (1917) we noted the occurrence of two such double infections. (Case E. 130 is one of these.) But we also noted that infections occur in which cysts of large and small size with numerous intermediates are present; and we left it an open question whether such a case represents a multiple infection, or an infection with a single strain producing cysts of very variable size. Cases E. 79 and H. 11 are two such instances; and the curves in Figs. 9 and 10 appear to us to demonstrate the fact that each possesses in reality a double infection essentially similar to that of Case E. 130. Careful measurements, so far as they have been made, have not yet furnished us with one instance of a single strain showing such great variation in the size of its cysts. We believe, therefore, that when cysts showing a great range of variation in size (e.g. 7$\mu$–15$\mu$) are present in a stool, a careful series of measurements of a sufficiently large number will probably reveal the fact that they belong to two—or possibly more—different strains.

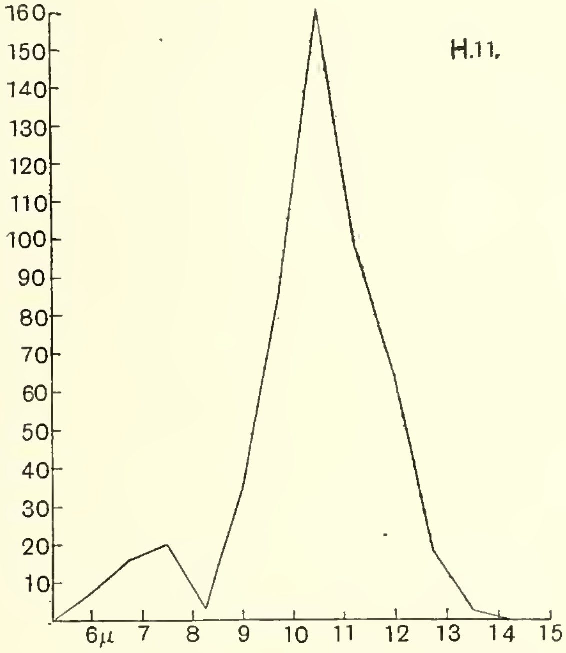

Fig. 7.

We have already given the calculated value for the commonest size of cyst occurring in each of the four simple infections which we have called H. 8, H. 7, E. 42, B. 1. If we deal in the same way with the three double infections just described, we obtain the following figures for the dimensions of the living cysts occurring with the greatest frequency:

| | |
|---|---|
| Case H. 11 ... ... | 8·3$\mu$ and 11·6$\mu$ |
| ,, E. 79 ... ... | 8·3$\mu$ and 11·6$\mu$ |
| ,, E. 130 ... ... | 6·6$\mu$ and 11·6$\mu$ |

On comparing these figures with those already given it will be seen that the smaller cysts in Cases H. 11 and E. 79 have the same diameter (8·3$\mu$) as those of Case H. 7; whereas the smaller cysts of Case E. 130 correspond in size (6·6$\mu$) to those of Case H. 8. The larger strains from all these cases are, however, though coinciding with one another, different in size (11·6$\mu$) from any of those which we have previously described. It appears, therefore, that the three double infections just described supply us with but one additional race. And hence we conclude that, in the stools of the seven patients studied, cysts belonging to five different races of *E. histolytica* were present. The approximate size of the living cysts occurring with the greatest frequency in each of these five races is as follows:

6·6$\mu$, 8·3$\mu$, 11·6$\mu$, 13·3$\mu$, 15$\mu$.

It must be expressly noted that the strains of *E. histolytica* just described constitute merely a selection from those which we have studied. We have no doubt that, had it been possible for us to make an equally detailed study of all the cases in our series, we should have found other races possessing cysts with other mean diameters. As already noted above, we have found the strains with cysts having diameters from about 11$\mu$ to 14$\mu$ to be the commonest, though strains resembling in size those of Cases H. 8 and H. 7 are by no means infrequent. We have seen, however, very few strains indeed having mean diameters, for the living cysts, of intermediate magnitude (about 10$\mu$). Of the 202 *E. histolytica* infections which we described in our earlier note (1917) there was but one of this character (Case E. 154)[1]. A case which appears to be of the same type has been recorded by Wenyon and O'Connor (1917—Case "Kettlewell," p. 160). Of the other cases described by these observers, we note that one is similar to our Case H. 7; three others resemble our Case E. 42; and the remaining one is of the type of our Case B. 1. The figures, as far as they go, of these authors thus agree closely with our own; and they have also concluded that "it may be safely stated that the majority of cases of *E. histolytica* infection show cysts with a diameter of ten to fourteen microns." At the present time, however, we are not so much concerned with an investigation of the actual number of strains of *E. histolytica* which may exist, or of their frequency, as with the demonstration that such strains actually do exist.

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[1] Unfortunately, we have been unable to make a large series of measurements of the cysts from this case. When the case was first examined, the size of the cysts—based on relatively few measurements—was recorded, and stained preparations were made for further study. On examining these later, however, we were disappointed to find that the cysts were too distorted for satisfactory measurements to be obtainable. (They were fixed in Schaudinn's fluid—without acetic acid.)

For the complete demonstration of this fact, however, it is necessary to prove that the mean diameter of the cysts from any patient is not subject to any considerable variation from day to day, but remains constant. That this is so we think nobody who has studied any number of *E. histolytica* infections for any length of time will be inclined to doubt. It is, indeed, a fact which one confirms almost daily in the course of routine examinations. A patient originally found to be passing cysts of a certain mean diameter is found later to be passing cysts of the same size whenever his stools are positive; and this applies both to untreated cases, and also to those who relapse after treatment with emetine. Case E. 42, for example, who was under observation for about seven and a half months, during which time he was treated and relapsed four times, always, when positive, passed cysts similar to those already described (mostly 12–14$\mu$ in diameter). We did not always measure a large number of cysts from his stools, but the measurements which we made were always closely similar. The two largest series of measurements of living cysts from this patient have already been recorded. Five hundred measured on May 21st, 1917, gave a mean diameter of approximately 13$\mu$, five hundred measured on June 23rd, 1917, after a prolonged course of unsuccessful treatment during the period between these dates, were found to have a mean diameter of 13·2$\mu$. Similarly another case (E. 149) which was originally found to be passing cysts 6·5$\mu$–8$\mu$ in diameter on January 16th, 1917, remained under observation until the end of May. During this period he was unsuccessfully treated with emetine three times, and at each relapse cysts of the same size were present in his stools. Observations similar to the foregoing could be multiplied almost indefinitely from our records. In fact it is a rule, to which so far we have found no exception, that the size of the cysts passed by any individual infected with *E. histolytica* remains constant as long as the infection persists.

It is to be noted, however, that a patient infected with two different strains at the same time may present an apparent exception to this rule. As is now well known, cysts are not always present in the stool of an infected person—or, at all events, are not always present in discoverable numbers. From time to time the stools are negative. Now it is clear that a person infected with two different strains may sometimes be negative for either or both. If, therefore, at a single examination cysts of large size are present, it cannot be concluded that the patient is infected with this strain only. At the next or at a later examination cysts of small size may be found, either alone or accompanied by the larger cysts. A careful study of such a case by more prolonged examination and the measurement of a large series of cysts will alone enable one to arrive at the correct interpretation. One of our cases (E. 58) furnishes us with a case in point. This man, when first examined, was found to be passing numerous cysts of *E. histolytica* of large size—mostly from $12\mu$–$14\mu$ in diameter. A second specimen, examined at a later date, contained cysts which at first sight appeared to be all of small size ($8\mu$–$9\mu$). A careful study of this stool, however, revealed the fact that large-sized cysts were also present in very small numbers; and a series of measurements clearly showed that we were dealing with a double infection similar to those which we have already described. The diameters of the two strains were $8\mu$–$9\mu$ and $12\mu$–$14\mu$. Cases such as this, therefore, though apparently indicating at first sight an inconstancy in the size of the cysts, in reality, when carefully studied, furnish most conclusive evidence of the constancy of the different strains of *E. histolytica.*

We believe, however, that certain conditions may, to a slight extent, temporarily modify the size of the cysts of a given strain. Although we can adduce no large series of measurements in support of our belief, we have often observed a slight increase in the size of the cysts from a patient just relapsing after unsuccessful treatment with emetine. These cysts are usually very degenerate, and frequently all uninucleate. In the case of the larger strains ($12\mu$–$14\mu$ before treatment) they may be $15\mu$ or more in diameter; such large cysts constituting the great majority—not, as originally, the rare exceptions—among the cysts in the stool. These large and degenerate cysts often give rise to considerable difficulties or uncertainties in diagnosis. We have invariably found, however, that, after a few days, cysts of normal size and structure reappear in the stools; thus enabling one not only to confirm the diagnosis, but also to verify the fact that the administration of emetine has brought about no permanent change in the size of the cysts.

Since it appears probable, from these observations, that a change in the environment of the amoebae—in the above case, a change produced by the administration of emetine to their host—may modify the size of the cysts which they produce, the suggestion might be made that the differences in size in the cysts from different patients may be due to comparable causes. It might be supposed, for example, that when one patient passes cysts with a diameter of $7\mu$, whilst another passes cysts of $12\mu$, this is not because they are infected with two different strains, but because the same strain, when living in two different environments (hosts), produces cysts of two different sizes. Unless such a possibility could be excluded, it is clear that we should not be completely justified in speaking of the different-sized cysts, in the infections which we have studied, as belonging to different strains or races of the parasite. It has not been possible to test this supposition by direct experimental infection of human beings by means of cysts of known sizes; but the question can nevertheless be answered almost conclusively, we believe, from some of the observations which we have already recorded. We think that we have demonstrated above that cysts of two different mean sizes may coexist in the stools of the same host (cp. Cases E. 130, E. 79, H. 11, *supra*); and this fact clearly indicates that the size of the cysts, whether large or small, is not determined by the host. It is of course conceivable, though we think highly improbable, that the site of infection in the large intestine might influence the size of the cysts produced by the parasites. But since we know no facts or analogies to support such an assumption it seems unnecessary to discuss it further. It seems to us, therefore, that we are justified in concluding (1) that the differences in mean diameter of the cysts passed by different persons are to be correlated with differences in the parasites themselves and not in their hosts; and (2) that these differences indicate that there are distinct groups of individuals among the parasites themselves which may properly be designated "strains" or "races" within the single species *E. histolytica*.

In concluding this section we may refer to a point of some practical importance connected with the recognition of the different strains of *E. histolytica*. It has been our custom for a considerable time to record the diameter of the cysts for every infection which we have studied. We have thus known with some degree of certainty with what strain or strains of *E. histolytica* each patient has been infected before beginning treatment with emetine. This knowledge has on several occasions proved useful subsequently. In dealing with a large number of military patients it is often difficult, and sometimes impossible, to make certain that every sample of faeces arriving at the laboratory has been passed by the particular patient whose name it bears. In spite of every precaution, substitution and interchange of specimens from time to time occur; and a case which is in reality negative may thus appear positive, and *vice versa.* On three occasions our patients have "relapsed" after treatment with emetine bismuth iodide, the "relapse" strain having cysts of a different size from those of the original infection. As such an occurrence appeared to us highly improbable in the light of the results given above, we were led on each occasion to challenge the authenticity of the specimens. A more rigorous control of the patients, and the continued examination of their faeces, ultimately proved in each case that our suspicions were justified. All three cases were subsequently found consistently negative, and were therefore finally discharged as cured. Had we not carefully studied and recorded the size of their cysts before treatment, each of these cases would, of course, have been wrongly classified as a relapse and re-treated.

## COMPARISON OF OUR CONCLUSIONS WITH THOSE OF SOME OTHER WORKERS.

Before discussing our results and their relation to those of certain other observers, it will be convenient rapidly to survey these results themselves. We have, indeed, already summarized them to some extent in our previous paper (1917), and at various points in the course of the present work. But it will greatly facilitate the ensuing comparison if we again summarize our chief conclusions at this point collectively.

Our chief result is that we have, as we believe, demonstrated that the human dysentery amoeba (*E. histolytica* Schaudinn) is a collective species, which includes a number of distinct races or strains of individuals which can be readily distinguished from one another by the sizes of the cysts which they produce. The cysts produced by the different strains differ from one another morphologically in no respect save size, being in all other respects indistinguishable. Whether these strains differ from one another in pathogenicity or in other physiological characters we have as yet no means of determining with certainty. Nevertheless, we have observed that all react in the same way towards treatment with emetine, and we think it probable that no such differences exist. We believe that we have been able to show with considerable probability that the different groups of individuals constituting these so-called "strains" really are such, since they appear to be permanent and not to be merely a result of differing environmental conditions. In other words we regard them as constituting so-called "petites espèces" (Jordan) or "pure lines"

(Johannsen) such as have been demonstrated to exist in almost all species of organisms which have hitherto been studied systematically[1].

We have obtained no evidence that races of *E. histolytica* producing cysts of any particular size are characteristic of infections acquired in any particular locality. We have found cysts of the commonest size ($11\mu$–$14\mu$) in carriers who contracted dysentery in all parts of the world from which we have examined cases (Europe, Asia, Africa, and America). The strains producing small cysts have been detected similarly in cases from many parts of the world (France, Egypt, Gallipoli, Salonica, Mesopotamia, India). Cysts of the larger diameters have been found in the stools of dysentery patients from Egypt, Gallipoli, Salonica, China, Thibet, Malay States, and S. Africa. It is not of course possible to say that the infection was acquired in every case when and where the attack of dysentery occurred, though this appears probable in many cases.

We have pointed out elsewhere (1917) that the name "*E. minuta*" is improperly applied to those races of *E. histolytica* which produce cysts of small size. We think it necessary to emphasize this once more, since the contrary has again been asserted by Woodcock (1917). He says "the name 'minuta' should be reserved for the distinctly small type or variety, the cysts of which are only about 8 microns in diameter." There can be no question however that the name *Entamoeba minuta* was given by Elmassian (1909) to the common race of *E. histolytica* producing cysts of large size (about $12\mu$ in diameter). It follows, therefore, that *minuta* is a synonym without validity for *histolytica*; and if the name "*minuta*" is retained for any stage in the development of this parasite, then it must be applied only to the small precystic amoebae, characteristic of subacute infections, which were described and figured by Elmassian and to which he gave the name.

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[1] Comparable instances in the Protozoa are to be seen in the two species *Paramecium caudatum* and *P. aurelia*. It has been shown by Jennings (1908) that within each of these species there are numerous strains which differ from one another only in the average size of their constituent individuals. It is true that in this case the characters distinguishing the pure lines from one another are those of the organisms themselves—not of their cysts. In the case of *E. histolytica* we are still uncertain whether the full-grown amoebae belonging to strains producing cysts of different size are themselves likewise distinguishable in the same respect. (The precystic "*minuta*" forms of the different strains undoubtedly correspond in size to the sizes of cysts which they produce. Cp. Plate VI, Figs. *E. h.* (2) and *E. h.* (3), and see also our earlier paper, 1917.) Nevertheless, whether this be the case or not appears to us immaterial, since the size of the individual or the size of the cyst might be used with equal propriety to discriminate races from one another. A summary of the literature bearing upon pure lines in the ciliate Protozoa has been published elsewhere by one of us (C. D. 1914).

It is of course possible to regard each race possessing cysts of a given size as a distinct variety which merits a distinctive varietal name, assigned in accordance with the rules of nomenclature. At present, however, it seems to us superfluous to invent such names, since we do not know how many different races actually exist, and since for those races already studied a simple numerical expression of the size of the cysts furnishes an adequate distinguishing mark.

From a practical point of view it is of course most important that the cysts of the various strains of *E. histolytica* should be recognized and accurately distinguished from those of the other amoebic parasites of the human bowel. This aspect of the matter has, however, already been considered by Wenyon and O'Connor (1917) and ourselves (1917), and need not be further discussed here.

As we have already noted more than once, our conclusions in general appear to agree very closely with those of Wenyon and O'Connor (1917); but this is by no means the case when we come to consider the results of some other workers who have published figures and statistics relating to the cysts of *E. histolytica*. Two such publications we shall consider in some detail.

1. *Kuenen and Swellengrebel* (1913). These authors have stated that the cysts of *E. histolytica* vary in size from 11 $\mu$ to 19 $\mu$. They give a curve which possesses a single apex situated at 13 $\mu$. With regard to these findings we will merely note the following points. First, the form of the curve obtained does not differ in any important respect from some of our own. Secondly, it seems clear that their figures apply, not to the whole species *E. histolytica*, but only to one of the commoner strains producing large cysts—the strains producing small cysts having escaped the authors' notice completely. And thirdly, it is clear that no far-reaching conclusions can be drawn from the study of a single strain, and of only a hundred cysts belonging to it—the number actually considered by these observers. Although at first sight, therefore, these results may appear very different from our own, we believe that they become easily reconcilable with them when the deficiencies just noted are taken into account.

2. *Mathis and Mercier* (1916, 1917).—In a series of papers these two authors have recently made and repeated a number of statements concerning the diameters of the cysts of *E. histolytica*; and have moreover drawn from their findings certain conclusions concerning the life cycle of the parasite. Their conclusions are briefly as follows (see especially Mathis and Mercier, 1916 *a*, p. 980, and 1917 *a*):

1. The cysts of *E. histolytica* possess diameters ranging from $10\mu$ to $15\mu$ (living cysts). They never pass these limits.

2. The majority of the cysts are *either* $12{\cdot}5\mu$ *or* $14{\cdot}0\mu$ in diameter. From 40 % to 45 % of the cysts in *every* infection fall under each of these categories—larger, smaller, and intermediate-sized cysts being exceptional.

3. The cyst population is thus dimorphic, consisting of "microcysts" and "macrocysts." It is supposed that, on being ingested by a fresh host, they liberate respectively microgametes and macrogametes.

Before considering these conclusions in detail we will briefly refer to the methods of investigation employed by these authors, whose results are so conspicuously different from our own. It is clear, both on general grounds and for particular reasons considered in the earlier part of the present paper, that, to obtain satisfactory results in an investigation of the present character, the greatest attention must be paid to methods and technique. It is regrettable, therefore, that the authors have not thought it necessary to give anything but the slightest indications in this respect. They tell us merely that their measurements have been made—they do not tell us how—on stained and mounted cysts; that with the technique which they employed (fixation with sublimate-alcohol-acetic acid, staining with iron-haematoxylin and eosin) the cysts undergo a contraction of $2\mu$ to $2{\cdot}5\mu$—for which a correction is made to determine the size of the living cysts; and, though no adequate figures are given, that the dimorphism can be made evident by measuring 200 cysts. The only curve which they give (1917 *a*) in illustration is, however, based upon the measurements of only one hundred.

Since the authors have not yet described (so far as we are aware) their methods of measuring cysts—which, as we have seen, is a by no means simple matter—it is impossible for us to judge whether the discrepancy between their findings and ours depends upon differences in the technique adopted. This lack of information becomes especially baffling when we come to consider the figures which they give for the differences in size between living cysts and those which have been fixed, stained, and mounted. The authors' method of fixation was similar to our own; and their method of staining sufficiently like ours to make it probable that it could not account for any considerable difference in the results. Their statement, however, that the process of fixation, staining, and mounting causes a cyst to undergo a reduction of $2\mu$ to $2{\cdot}5\mu$ in diameter is completely opposed to our findings. Since we have devoted much attention to this matter, and all our findings have been perfectly consistent, we can only suppose that the authors are mistaken in their assertion; and that, in the absence of any indication to the contrary, we are justified in concluding that the lightness with which they dismiss this difficult problem reflects the spirit in which they effected its solution.

It seems clear, from their published statements, that Mathis and Mercier have, like Kuenen and Swellengrebel, ignored all strains of *E. histolytica* save the commonest—those which, as in our Case E. 42, produce cysts having a mean diameter between 12$\mu$ and 14$\mu$. The smaller and larger strains they have, apparently, completely overlooked.

The curve which Mathis and Mercier have published (1917 *a*, p. 168), and upon which the proof of a dimorphism in the cysts of *E. histolytica* appears to rest, is to us wholly incomprehensible. As we have seen, the measurements which we have made of single strains of *E. histolytica* have consistently given us unimodal curves. There has always been a common diameter for the majority of the cysts, with the minority grouped more or less symmetrically above and below it. Even by taking so few as a hundred cysts we cannot understand how it has been possible to obtain two modes separated from one another by only 1·5$\mu$, with only half-a-dozen cysts of intermediate dimensions. In no instance have a hundred cysts taken at random from our series of measurements given us a curve of this form. We were at first inclined to suppose that the dimorphism was similar to that which we ourselves have found in Case E. 130—that is to say, it resulted from an infection with two different strains. This explanation, however, will not suffice: for the superposition of the curves from two different strains having their maxima at 10$\mu$ and 11·5$\mu$ would not give a curve like that of Mathis and Mercier. It would be necessary to assume in addition that the strain having the larger cysts showed practically no variation in size below the mean, while the smaller showed none above. Another possible explanation is that which we have suggested (*supra*, Part I, A) to account for the anomalous bimodal curve which we obtained from one of our own series of measurements.

Whatever the correct interpretation may be, it is clear from our own measurements that there are many strains of *E. histolytica* whose cysts display no discoverable dimorphism in respect of size. Indeed, it appears to us that when a dimorphism of the cysts in any given stool is demonstrable, it is to be interpreted as the result of a simultaneous infection of the patient with two different strains. This very obvious interpretation is not even considered by Mathis and Mercier, though no proof that there is a dimorphism of the cysts in any—still less in every—strain can be furnished until this possibility has been excluded. For our own part we can only suppose, in the light of our own results, that the conclusions of Mathis and Mercier rest upon some fallacy in observation or interpretation which, in the absence of all data for its determination, we are at the moment unable to define.

Although we believe that the conclusion of Mathis and Mercier that the cysts of *E. histolytica* are constantly differentiated into "microcysts" and "macrocysts," destined to liberate microgametes and macrogametes respectively, is unsupported by facts, we would not be understood to maintain, either that the small amoebae which emerge from the cysts are not gametes, or, if they are, that they do not belong to two different categories. In the absence of any evidence save a somewhat vague analogy with other organisms, we think it is as yet unprofitable to discuss such hypotheses further. We may, however, point out that, far from denying a sexual development in *E. histolytica* at the stage in the life-history and of the kind postulated above, this very suggestion was, we believe, made originally by one of us (C. D., 1909) as a result of a detailed study of the very closely related species *E. ranarum*.

### SUMMARY AND CHIEF CONCLUSIONS.

1. *Entamoeba histolytica* Schaudinn (vel *E. dysenteriae* Councilman and Lafleur), the human dysentery amoeba, is a collective species. It comprises a number of distinct races, strains, or pure lines, distinguishable from one another by the size of the cysts which they produce.

2. How many such distinct races exist is still undetermined; but we have demonstrated the existence of at least five.

3. There is no evidence that the different races differ in their geographical distribution, or in any character save size.

4. These races remain constant in character within a given host; and the dimensions of the cysts are not determined by the action of the host upon the parasite, since two different races may coexist side by side in the same host.

5. Different values for the dimensions of the cysts are obtained when these are measured in different media. Taking the measurements of living cysts suspended in physiological saline solution as representing the true value, we find that the dimensions of cysts in iodine solution are approximately the same; but that cysts which have been suitably fixed, stained, and mounted in balsam, have undergone an apparent reduction of approximately 10 % from the true diameter.

6. The most suitable fixative for *E. histolytica* cysts we have found to be a modification of Schaudinn's fluid. (Saturated aqueous solution of

corrosive sublimate, two parts; absolute alcohol, one part; glacial acetic acid, 4–5 %.) Cysts fixed in this fluid undergo no measurable shrinkage or swelling in the process.

7. The apparent loss of size observed in cysts which have been fixed, stained, and mounted in balsam, is due chiefly to the invisibility of the cyst wall in the mounting medium.

8. It follows from what has been said above (5 and 6) that to obtain an approximately true value for the dimensions of the living cysts from measurements made upon those suitably fixed, stained, and mounted in balsam, these measurements should be multiplied by the factor $\frac{10}{9}$.

9. We find no evidence of the existence of a sexual dimorphism in the cysts belonging to a single race, such as has been described by Mathis and Mercier.

10. Since cysts of *E. histolytica* may be found with all diameters from about $5\mu$ to about $20\mu$—a range overlapping or covering the dimensions of the cysts of the two common harmless intestinal amoebae of man (*E. coli* and *E. nana*) and of other cysts and cyst-like bodies occurring in human faeces—it is of considerable practical importance to recognize and distinguish the diverse races of *E. histolytica* which differ from one another in the dimensions of the cysts which they produce.

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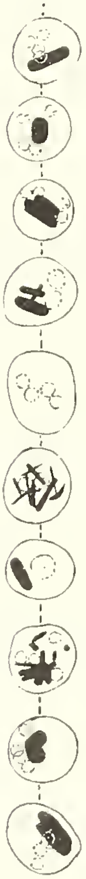
H.8.



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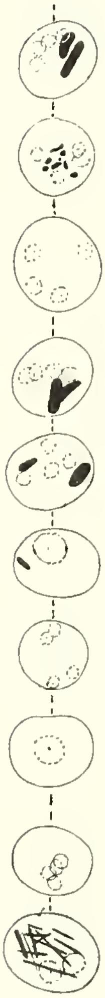

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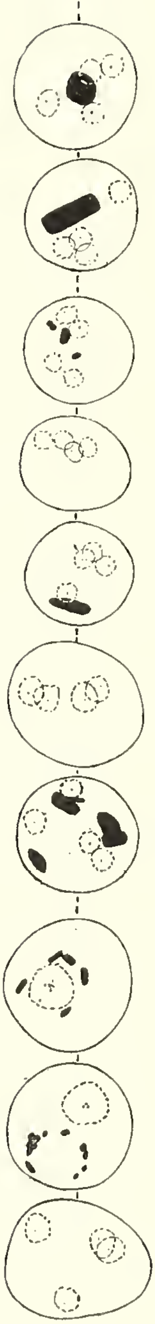
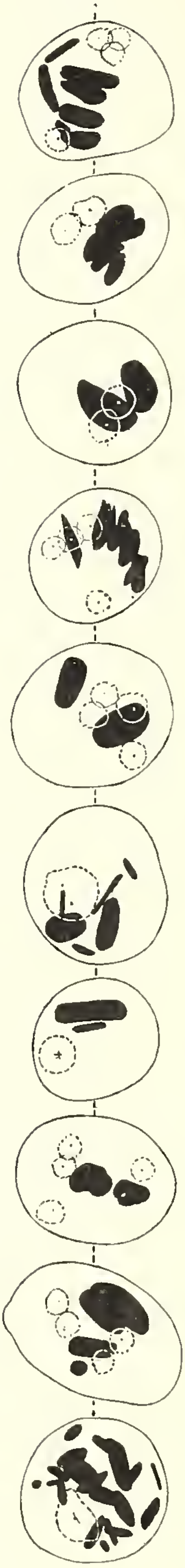
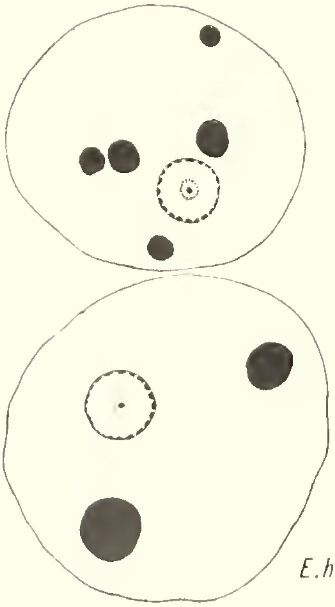


B.1.





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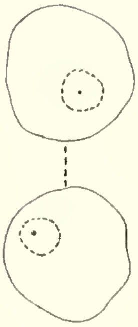

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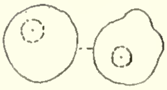

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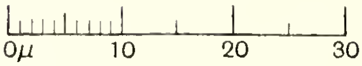

C. D. del.

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## DESCRIPTION OF PLATE VI.

The figures in this plate have been drawn to show at a glance the differences in the dimensions of the cysts of *E. histolytica* in the four cases (H. 8, H. 7, E. 42, B. 1) described in the text. They show in parallel columns ten cysts from each of these cases. The cysts were not selected, but represent in each case ten consecutive cysts in the order in which they appeared in examining the preparations at random under the microscope. The preparations were all fixed, stained, and mounted in the manner described in the text. The drawings show merely the outlines of the cysts, their nuclei, and chromatoid bodies (black) when present. The outlines were drawn as accurately as possible with the camera lucida, at a magnification of 2500 diameters (Zeiss 2 mm. apochromat, N.A. = 1·40, compensating ocular 18), and have been reduced to the size shown in the process of reproduction. The figures illustrate not only the differences in the dimensions of the cysts in the four races of *E. histolytica* described, but also the kind of asymmetry observable in the outlines of the cysts themselves, which renders a perfectly accurate determination of their diameters extremely difficult.

The remaining figures are outlines of fixed and stained amoebae, drawn in the same way to the same scale, to illustrate the size of the unencysted organisms as compared with their cysts. Fig. *E. h.* (1) shows two individuals of the tissue-inhabiting form ("*histolytica*" form) of *E. histolytica*, from a case of acute dysentery. The amoebae belong to a race producing cysts similar in size to those of Case E 42. Fig. *E. h.* (2) shows two small precystic amoebae ("*minuta*" forms) from a subacute infection, and belonging also to a race of *E. histolytica* having cysts of similar size. Fig. *E. h.* (3) shows a pair of precystic amoebae ("*minuta*" forms) belonging to a race with cysts similar in size to those of Case H. 8. (The black masses in Fig. *E. h.* (1) are the remains of ingested red blood-corpuscles.) The amoebae were not specially selected individuals, but were taken at random from preparations in which large numbers were present.

# *DIENTAMOEBA FRAGILIS* N. G., N. SP., A NEW INTESTINAL AMOEBA FROM MAN.

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*Imperial College of Science.*

(*A REPORT TO THE MEDICAL RESEARCH COMMITTEE*[1].)

(With Plate VII.)

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## INTRODUCTION.

It is now becoming generally recognized that there are three different amoebae which may be present in the human bowel—the harmless *Entamoeba coli* (Lösch) Schaudinn and "*Entamoeba*" *nana* Wenyon and O'Connor, and the pathogenic *E. histolytica* Schaudinn. We have discussed these three species in an earlier paper (1917), and there noted their chief distinctive characters. In the present paper we shall describe a fourth and much less common species which we have recently encountered. It differs in some respects so conspicuously from the others that it appears to us necessary to place it not only in a new species, but even in a new genus. We propose therefore the new name *Dientamoeba*

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[1] The observations here described were chiefly made by us whilst working at the Wellcome Bureau of Scientific Research, and with the aid of grants from the Medical Research Committee. We take this opportunity of recording our indebtedness to the Bureau for the facilities and assistance which it has afforded us in the course of our work.

*fragilis* for our amoeba, but we can discuss the question of nomenclature most conveniently after we have described the organism itself. This, therefore, we shall now do.

## DESCRIPTION OF THE AMOEBA.

The amoeba is a very small organism, in the living state somewhat resembling "*Entamoeba*" *nana* in size and general appearance. Living individuals, when rounded, measure as a rule about $8\mu$ to $10\mu$ in diameter, though larger forms (up to $12\mu$) also occur, and extremely minute individuals (down to $3{\cdot}5\mu$) are not infrequent. Usually the dimensions are slightly less in fixed and stained organisms.

The living amoeba moves actively by means of pseudopodia which usually differ in form from those of "*E.*" *nana*, *E. histolytica*, and *E. coli.* The pseudopodia are extremely thin, hyaline, and leaf-like, and the ectoplasm of which they are composed is separated by a fairly sharp line of demarcation from the granular endoplasm of which the rest of the body mainly consists. (See Plate VII, figs. 1–3.) The broad flat pseudopodia are often irregularly lobed or marginally indented (Fig. 1). At times they suggest comparison with a bat's wing; or when, as is not uncommon, they display three pointed lobes, their shape recalls that of an ivy-leaf. In progression the pseudopodia are confined to that side of the organism which is anterior; the rest of the body, containing the bulk of the protoplasm and its inclusions, remaining as a dense, rounded, posteriorly placed mass. Such individuals, when in active movement, somewhat resemble little snails (cf. Fig. 2).

The endoplasm is finely granular and colourless in the living amoeba. It usually contains numerous food vacuoles enclosing small bacilli and cocci. The fixed and stained organisms show a distinct alveolar differentiation of their cytoplasm (cf. Figs. 4, 5, etc.): but healthy living individuals, as a rule, possess no vacuoles save those containing food. There is no contractile vacuole. In iodine solution the amoebae sometimes show diffuse brown-stained patches, probably indicating the presence of glycogen: but whether this is contained in vacuoles or is in solution in the protoplasm we have not been able to determine.

By far the most characteristic feature of the amoeba is its nuclear system. As a general rule the individuals are binucleate (cf. Figs. 4, 5, 10, 11, 13). The nuclei are usually invisible in the living organism; but in stained specimens they are easily visible, and are seen to be exactly alike in size and structure (cf. Figs. 4, 5, etc.). In an individual of average size the nuclei are about $2\mu$ in diameter: but as a general rule they are proportionate in size to the size of the whole organism, and range in diameter from about 2·3$\mu$ in very large to 0·8$\mu$ in very small individuals (cf. Figs. 5 and 13).

Although most individuals are binucleate, some can always be found which are uninucleate. Such individuals occur among amoebae of every size, but are commonest among those of small and intermediate dimensions (see Figs. 9, 12, 14). We have endeavoured to determine the proportions of binucleate and uninucleate amoebae in the species by counting the number of individuals of each category in preparations of the stools of the same person on different days, and in preparations made from different cases. The proportions found on different occasions are given below in tabular form:

| Case No. | Date | No. of amoebae with 2 nuclei | No. of amoebae with 1 nucleus | Total No. counted | Percentage of uninucleates |
|---|---|---|---|---|---|
| D. 1 | 27. i. 17 | 235 | 70 | 305 | 22·9 |
| ,, | 20. iv. 17 | 151 | 49 | 200 | 24·5 |
| D. 2 | 24. ii. 17 | 162 | 38 | 200 | 19·0 |
| D. 4 | 9. vii. 17 | 162 | 38 | 200 | 19·0 |
| ,, | 11. vii. 17 | 170 | 30 | 200 | 15·0 |
| | Totals: | 880 | 225 | 1105 | 20·3 |

It will be seen that there is a good deal of difference in the percentages found; and it is possible that the greater number of uninucleates observed in case D. 1 on both occasions has some significance. However, the number of individuals counted at any single time is not great. Nevertheless, we think that the aggregate result justifies us in concluding that, on the average, about 80 per cent. of the individual amoebae in the whole population are binucleate and 20 per cent. uninucleate. The interpretation of this conclusion will be discussed below.

Although, as already noted, the structure of all nuclei appears to be the same, the appearances which they present may differ according to the way in which they have been stained. Each nucleus contains a large central karyosome whose diameter is about three-fifths of that of the entire nucleus. The nuclear membrane is extremely delicate, and separated from the karyosome by a clear zone traversed by a few very fine radiating linin threads (see Figs. 4, 9, etc.). There appears to be no chromatin on the nuclear membrane or in the clear zone, but sometimes excessively minute and faintly stained granules can be made out at the points where the radiating threads join the nuclear membrane. The karyosome, when deeply stained with iron-haematoxylin, appears almost homogeneous; but in specimens from which the stain has been extracted to a greater extent, and in those stained with paracarmine, haemalum, or by other progressive methods, it is seen to consist of a number of distinct granules of chromatin probably embedded in a plastin matrix (see Figs. 4, 5, 9, 10, etc.). Frequently one of these chromatin granules appears larger and more deeply stained than the others. Sometimes also a separate granule can be seen lying at the centre of the karyosome (cf. Fig. 9); but for reasons which have been given by one of us elsewhere (C. D., 1914), we shall not call this, or any other granule in the karyosome, a centriole. From the evidence before us we have no means of judging whether a centriole is present or not. In binucleate individuals the nuclei vary in their relative position. Sometimes they are in contact (Figs. 4, 13), sometimes separated to a greater or less extent (Figs. 5, 6, 10, etc.).

The food of the amoeba, judged from the inclusions in the food vacuoles, consists entirely of small bacteria and yeasts living in the intestinal contents.

The exact habitat of the organism in the intestine we cannot state with certainty, but the following observations indicate its probable location. One of our cases is infected with *Lamblia* as well as with *Dientamoeba*, and two others with *Entamoeba coli*. *Lamblia*, of course, lives as an active flagellate in the small intestine, only its cysts being found as a rule in the colon; whereas both the free amoebae and the cysts of *E. coli* are found in the large intestine alone. *Dientamoeba* is present in the stools of an infected person only when they are soft or diarrhoeic. At such times it is accompanied by the active forms of *E. coli*, in the cases infected with this organism; but by the cysts—not the flagellates—of *Lamblia* in the case harbouring this protozoon. We conclude from this that the amoeba occupies a site in the intestine where it is accompanied by free *E. coli* and encysted *Lamblia*; in other words, that it probably lives in the colon.

One of the most characteristic features of the amoeba is its extreme frailness after it has left the body—a character which suggested the specific name which we propose below. As a rule the majority of the individuals in a stool become rounded and begin to degenerate almost immediately. Unless examined on a warm stage, they rapidly cease to move, and die. The process of degeneration is so characteristic, and the degenerate individuals are so often the only forms present when the faeces of infected persons are examined, that we shall describe them in detail. Usually the first sign of degeneration in the living organism is the appearance of a vacuole in the protoplasm. This vacuole often becomes very large, and is generally lenticular or fusiform in outline (cf. Fig. 6). Some individuals develop numerous vacuoles, and thus appear to be full of bubbles. The vacuolate amoebae may continue to move for some time, but finally they all become rounded and die. By an increase in the size of the large vacuole or a fusion of smaller ones, the whole organism soon presents the appearances shown in Figs. 8 and 7. At this late stage in degeneration the whole amoeba has become one inflated vacuole surrounded by a thin covering of protoplasm, so that it appears annular in optical section (Fig. 7). Such ring-like individuals often measure $12\mu$ to $13\mu$ in diameter. The two nuclei can usually be seen in the unstained organism at this stage. In the faeces the degenerate ring-like individuals may remain almost unchanged for many hours, and sometimes some of them persist thus for even two or three days. At first sight they resemble dead *Blastocystis*, for which they can easily be mistaken. The large central vacuole of the degenerate *Dientamoeba* corresponds in position to the large central reserve body of *Blastocystis*, but has, of course, quite a different appearance. The resemblance, therefore, is most striking when the reserve body has disappeared from the dead *Blastocystis*, and its position is occupied by an apparently empty space. The nuclei sometimes break up before the *Dientamoeba* becomes ring-like, and may almost have disappeared before it ceases to move. In any case they undergo fragmentation sooner or later.

On account of the rapidity with which it degenerates and dies the living amoeba can only be studied satisfactorily in very fresh stools, and preferably on a warm stage. For the same reason good stained preparations can be made from such stools only; and even in the freshest specimens the amoebae are often very troublesome to stain, and many may appear degenerate.

It has recently been stated by Swellengrebel (1917) that *Blastocystis* is merely a degenerate form of several different intestinal protozoa. From our own observations—more especially from those of one of us (C. D.) which extend over more than ten years, but which are still for the most part unpublished—we have no doubt that he is mistaken. We have convincing reasons for believing that *Blastocystis* is not a degenerate protozoon, but an independent organism belonging to the vegetable kingdom. When, therefore, we refer above to the resemblance of a degenerate *Dientamoeba* to a dead *Blastocystis*, we would not be understood to suggest that they are in any way connected. Although most of our cases infected with *Dientamoeba* are infected also with

*Blastocystis*—which can, indeed, be found, if carefully sought, in most human beings—we have, we believe, in the present series one case (D. 4) which is uninfected with this organism. We do not, of course, deny that degenerate protozoa may be mistaken for *Blastocystis*; on the contrary, we believe that this is the key to the correct interpretation of Swellengrebel's observations.

Although we have devoted much time to the search, we have never been able to discover any other stages of *Dientamoeba* than the free forms described above. No cysts, or stages resembling cysts, have ever been found. We thought at first that we should have no difficulty in discovering the cysts if we continued to examine the faeces of an infected case for a sufficiently long time. All our hopes, however, have been disappointed. One case (D. 1) has now been examined 42 times during a period of ten months, and a most careful search for cysts has been made by both of us on very many occasions. Another case (D. 4) was examined with equal care 12 times in five weeks: but neither in the stools of these cases nor in those of any others infected with this amoeba have we succeeded in finding any structures which can be regarded as its cysts. When the stools of an infected person are soft or diarrhoeic—either naturally, or after the administration of a saline purge—the active amoeba can often be found in them: but when the stools are hard and formed no trace of its existence can be detected. In this respect *Dientamoeba* differs conspicuously from the other human intestinal amoebae[1], and resembles the flagellate *Trichomonas hominis*. We, like other observers, have never succeeded in finding the cysts of this organism, in spite of the most diligent search.

These two organisms—*Dientamoeba* and *Trichomonas*—differ remarkably in this character from all the other intestinal protozoa of man. Both present, in consequence, the same difficulty in the way of understanding their mode of transmission from host to host. It is possible, of course, that both form cysts or resistant bodies of some sort, and that these are formed very rarely or have been overlooked or mistaken for other structures. We find it difficult, however, to believe that these suppositions offer adequate explanations of the apparent invariable absence of cysts or cyst-like structures. It has been suggested by Wenyon

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[1] *Dientamoeba* resembles, however, in this respect another amoebic inhabitant of the human body—*Entamoeba gingivalis*, the amoeba commonly found in the mouth. Although "cysts" of this organism have several times been found and described, we have found no good evidence that the structures so interpreted are really the cysts of the amoeba. We believe, accordingly, that its cysts—if it forms any—have still to be discovered.

(1907) that an unencysted *Trichomonas* can pass through the stomach, and infect a new host in this way. We can hardly suppose, however, that *Dientamoeba* can thus effect its entry into the human body; for its fragility and lack of resistance when outside the body constitute one of its most characteristic features[1]. It occurs to us that there is another possible explanation of the apparent absence of cysts in these two organisms. It is well known that *Entamoeba histolytica*, which is probably a true human parasite, encysts in the typical manner within its hunan host. But it is also facultatively parasitic in other animals—for example, in the cat. In this host, however, the amoeboid form of the parasite alone occurs. The amoebae appear to have lost their power of forming cysts. It seems to us conceivable that a similar phenomenon may possibly by presented by *Dientamoeba* and *Trichomonas*. It is possible that both are parasites belonging to some host other than man, but capable of existing and multiplying in the human bowel. On such a hypothesis we would suppose that both organisms undergo encystation[2] in their normal hosts in the usual way, and that man becomes accidentally infected by ingesting their cysts. But in man, their foreign host, they have both lost—like *E. histolytica* in the cat—the power of forming cysts.

We may add here that we have made several attempts to cultivate *Dientamoeba* on agar media, but, as we expected, without success.

We have no reason to suppose that *Dientamoeba* is a pathogenic organism. It is true that we have found it chiefly in persons who have suffered from dysentery or chronic diarrhoea. This, however, ceases to have any significance when it is remembered that the vast majority of persons whose faeces we examine are so examined solely because of their intestinal ailments. Up to the present we have found only seven cases of infection with *Dientamoeba*, out of a very large number of persons whose stools we have examined. And although six members of our series are persons who have suffered from diarrhoea or dysentery abroad, on the other hand the remaining case (D. 1) is a person who has never left the British Isles, and who has never suffered from dysentery, persistent diarrhoea, or other intestinal ailment. We have endeavoured for some time past to examine, whenever possible, the stools of healthy persons in England in order to obtain some conception of the incidence of protozoal infections in such cases. The infection of Case D. 1 was discovered in the course of this investigation. We have so far discovered so few infections with *Dientamoeba* among the large number of dysenteric patients examined, that the single case among the non-dysenteric persons actually gives a higher percentage incidence in the latter class. Moreover, the mode of nutrition of *Dientamoeba* resembles that of *Entamoeba coli* and "*E.*" *nana*. It does not appear to be a tissue parasite like *E. histolytica,* and from this it may be inferred that it is, like the former, a harmless commensal. In contrast, however, with this supposition we must point out that three of our seven cases infected with *Dientamoeba* have histories of intestinal troubles of long standing, as a cause of which no concomitant protozoal or bacterial organism has yet been incriminated. (See cases D. 5, D. 6, and D. 7 below.)

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[1] *Trichomonas hominis*, on the other hand, will sometimes survive apparently unchanged for a long time after it has left the human body. On one occasion we found living and apparently healthy trichomonads in a sample of human faeces every time it was examined for four weeks after it was passed.

[2] Some species of *Trichomonas* undoubtedly form cysts. These were first described by one of us (C. D., 1908, 1909) in *T. batrachorum*, a species occurring in amphibians.

We may add that we can give no information concerning the action of emetine or other drugs on *Dientamoeba* infections, since none of our infected cases has undergone specific treatment.

It is only during the present year that we have recognized *Dientamoeba* as an independent organism. For this reason, and also on account of the rapidity with which the amoebae degenerate and disappear from the stools, we are at present unable to give any accurate figures for the frequency of its occurrence. Our impression is that it is very uncommon, but by no means so uncommon as our record of only seven infections in several thousand cases examined might appear to indicate.

We have been unable to find any reference to the organism which we call *Dientamoeba fragilis* in previous publications dealing with the intestinal amoebae of man or other animals; but we believe that it was previously observed, but not described, by Wenyon in 1909. Whilst in England recently (June, 1917) Colonel C. M. Wenyon showed us some preparations which he made eight years ago, and which contained, we believe, the amoeba here described. Colonel Wenyon was not aware, when he showed us his preparations, that we had independently recognized this organism at the beginning of 1917; but on seeing some of our preparations he agreed with us that his forms were probably the same as ours, and the priority of their discovery belongs, therefore, to him. We may add that we ourselves both discovered the organism independently of one another—one of us (M. W. J.) having found it about a month before the other (C. D.), who did not know of the discovery, at a time when we were not working in collaboration.

## DISCUSSION OF THE NUCLEAR SYSTEM OF *DIENTAMOEBA*. NOMENCLATURE.

Before we discuss the nomenclature of the organism here described, it is necessary to consider its nuclear system, and to compare it with certain other amoebae: since our interpretation of the nuclei, when contrasted with the nuclear structures of related forms, constitutes the chief justification for our regarding the organism as new, and consequently for proposing for it a new name.

We have already pointed out that most individuals of *Dientamoeba* are binucleate, though in a certain number (about 20 per cent.) only one nucleus is present. It might be suggested that this is due to the fact that, in the samples of infected faeces studied, the individuals—which are, in the resting stage, normally really uninucleate—happened to be encountered at a moment when rapid multiplication was taking place. We cannot accept this interpretation for the following reasons. First, although binucleate individuals are always in the majority, stages of nuclear division are either excessively rare or absent. That rapid division was occurring when the specimens were obtained appears therefore to be excluded. Secondly, binucleate individuals are always present in large numbers, no matter when the specimens are obtained. Whenever *Dientamoeba* is present in the stools, whether passed normally at any time or after the administration of a saline purgative, there is always the same preponderance of binucleate individuals. When the difficulty of obtaining dividing forms of any of the other intestinal amoebae by random sampling of the stools is remembered, it seems incredible that dividing forms of *Dientamoeba* should be present always. Thirdly, the binucleate individuals of *Dientamoeba* are actively motile (cf. Fig. 5); whereas most amoebae, when undergoing division, are rounded and at rest (cf. Dobell, 1914). Fourthly, it is to be noted that the binucleate or uninucleate condition is not peculiar to individuals of any particular size. If a uninucleate species is examined at the moment when an epidemic of division into two is occurring, the large dividing forms alone are binucleate, whilst the young forms resulting from the division are uninucleate. Here again, therefore, the hypothesis that *Dientamoeba* is an amoeba in process of division appears inadequate.

If the foregoing considerations appear unconvincing, and it be still urged that the organism is really only a division form of one of the amoebae of the human gut, then we would ask: To which species of human intestinal amoeba does it belong? Clearly not to *E. coli* or

*E. histolytica*, because of its very different size and structure. And although "*Entamoeba*" *nana* might from its size seem a plausible guess, a comparison of the nuclei of this species with those of *Dientamoeba* renders such a suggestion highly improbable; and the invariable absence of cysts from the faeces of persons infected with the latter alone, makes it impossible. It is clear therefore that, whatever the correct interpretation of the binucleate condition of *Dientamoeba* may be, we are dealing with an organism which is different from those hitherto described.

The interpretation which we put upon our findings is as follows. *Dientamoeba* is an amoeba which, when fully differentiated, possesses two nuclei. It appears to us probable that when an adult binucleate individual divides, it undergoes fission of the cytoplasm only, so as to form two small uninucleate individuals. The nucleus in each of these undergoes division into two at some time, which is variable, during the period of growth into the adult form.

Such a hypothesis will square with all our observed facts—with the preponderance of binucleate individuals in the population, and with the occurrence of binucleate and uninucleate amoebae of all sizes. Unfortunately we have found very few amoebae indeed in which nuclear division appears to be occurring. One such individual is shown in Plate VII, fig. 15, but from the very few nuclei that we have seen in division, we are unable to describe the process in detail. All the dividing nuclei have been seen in uninucleate individuals. We have never seen a binucleate individual with its nuclei dividing simultaneously, although we have seen two amoebae each containing four nuclei. We regard these quadrinucleate individuals as abnormal forms, similar to the binucleate and multinucleate individuals which occur occasionally in cultures of some of the small free-living amoebae resembling "*Amoeba limax*," or to the binucleate individuals sometimes seen in the three uninucleate intestinal amoebae of man. Such division stages as we have seen in *Dientamoeba* agree, therefore, with the interpretation outlined above.

Several binucleate amoebae are, of course, already known. The first, "*Amoeba*" *binucleata*, was described by Gruber (1885) from fresh water, and studied later by Schaudinn (1895), who described its method of nuclear division. It may be remarked that this organism can hardly be left in the genus *Amoeba*, though its transference by Doflein (1909) and Penard to the genus *Pelomyxa* appears to us equally impossible, and we believe that a new generic name must ultimately be assigned to it[1].

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[1] Alexeieff (1912, 1912 *a*) has proposed to place it, with other forms, in the genus *Hartmannella* Alexeieff (=*Hartmannia* Alex. 1912, preoccupied). We cannot agree with

Another free-living binucleate amoeba, "*Amoeba*" *diploidea*[1], has been described by Hartmann and Nägler (1908) and Nägler (1909). This organism again appears to us improperly placed in the genus *Amoeba*, on account of its nuclear arrangements and life-history. It also, we believe, will have to be placed in another genus, but one different from that occupied by "*Amoeba*" *binucleata*. Alexeieff (1912 *b*) proposes to place it in the genus *Sappinia* (Dangeard, 1896), a genus containing the species *S. pedata*, originally assigned to the Acrasiae. We believe that this proposal has much to recommend it, although it has been rejected by Chatton (1912). When, however, Alexeieff proposes further to place "*Amoeba*" *mira*, described by Gläser (1912), in the same genus, we are not able to agree with him completely: for we cannot determine whether this name was given to a uninucleate or a binucleate form. It should be noted that "*A.*" *binucleata* and "*A.*" *diploidea* appear to divide in a somewhat similar manner. In both, division of the two nuclei takes place before fission of the cytoplasm, so that the organisms—both young and old—are always binucleate[2]. The genus *Paramoeba* was proposed by Schaudinn (1896) for a marine amoeba possessing two "nuclear" structures; and some parasitic amoebae, originally described in *Sagitta* by Grassi, have recently been placed in the same genus by Janicki (1912). The latter amoebae appear to resemble, both as regards life-history and nuclear structure, the form described by Schaudinn; but his observations and also those of Janicki appear to us still problematic. In any case the organisms which they studied are forms possessing two nuclei—if they really are both nuclei—which are unlike; and it is clear that our new amoeba cannot be placed in the genus *Paramoeba*[3].

The foregoing considerations will make it evident that the binucleate

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him in this, as we believe "*Amoeba*" *binucleata* should be placed in a genus different from that occupied by the type species of the genus *Hartmannella* (*H. hyalina* Dangeard).

[1] This organism was obtained by Hartmann and Nägler in cultures from the faeces of lizards. We ourselves have found it on one occasion in a sample of human faeces, in which it made its appearance after the specimen had been kept in a glass tube for some days in the laboratory.

[2] Nägler (1909), however, notes the occasional occurrence of uninucleate individuals in "*A.*" *diploidea*—an observation which we are able to confirm from our own study of this organism.

[3] Some organisms found in the intestine of man have been placed in the genus *Paramoeba* by Craig (1906), and later in the genus *Craigia* by Calkins (1912). They have since been recognized by Barlow (1915). The forms described by these authors have nothing to do with the organism which we call *Dientamoeba*, nor can they be placed in the genus *Paramoeba* Schaudinn. They are not binucleate amoebae, so far as we can determine, and owe their existence, in our opinion, to errors of observation and interpretation. We regard "*Craigia*" as a non-existent organism and the genus as *nomen nudum*.

amoebae hitherto described differ conspicuously from the form which we have found in man. It seems to us clear, moreover, that our amoeba cannot be placed in the genus *Entamoeba* (= *Endamoeba*). The type species of this genus is *E. blattae* (Bütschli) Leidy 1879, an organism very different from the one under consideration. And although the three known intestinal amoebae of man are now usually placed in the same genus, there is reason to suppose that they may ultimately have to be separated, as they all differ considerably—so far as can be judged at present—from the parasite of the cockroach. Whilst, however, "*Entamoeba*" *histolytica* and "*E.*" *coli* are undoubtedly co-generic[1], it is not improbable that "*Entamoeba*" *nana* is generically distinct. But for our present purpose it does not matter how the genus *Entamoeba* may ultimately be revised or amended. It is sufficient to note that in any case it cannot properly be made to comprise the form which we have just described. The nuclear structure of the latter is alone sufficient to distinguish it sharply from any organism which can be regarded as the type of the genus *Entamoeba*.

We therefore think it necessary to introduce a new generic name for the organism which we have described: and accordingly we propose the name *Dientamoeba*—a name suggested by the nuclear structure, which is duplicate in comparison with that of other parasitic amoebae of the genus *Entamoeba*. A diagnosis of the new genus and species may now be given:

*Dientamoeba* nov. gen.—Small amoeba, with a diameter when rounded of ca. $3{\cdot}5\mu$ to $12\mu$ (average ca. $9\mu$). Cytoplasm differentiated into ectoplasm and endoplasm. Pseudopodia flattened, hyaline, and leaf-like; usually few, with irregularly dentate margins. Progression snail-like. No contractile vacuole. Typically binucleate, both nuclei having the same size and structure. Size of nuclei ca. $0{\cdot}8\mu$—$2{\cdot}3\mu$ (average ca. $2\mu$). Each nucleus possesses a large central karyosome surrounded by a clear zone containing no peripheral chromatin and limited externally by a very delicate achromatic nuclear membrane. Details of nuclear division unknown; but organism probably divides when binucleate into two uninucleate daughter-individuals, which become binucleate by subsequent division of the nucleus during growth. Cysts unknown. Habitat: intestine (probably colon) of man. Food: small vegetable organisms in intestinal contents.

Only known species *D. fragilis* nov. spec., with characters of genus.

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[1] If these two species are ultimately placed in another genus, then it must be *Loeschia* (Chatton and Lalung-Bonnaire, 1912), not *Proctamoeba* (Alexeieff, 1912).

## NOTES ON INFECTED CASES.

Since we believe that this is the first description which has been given of the organism which we call *Dientamoeba fragilis*, we will record briefly the histories, so far as they are available and have any present interest, of the infected cases which we have studied. We are indebted to Major J. B. Stephens, R.A.M.C., for information concerning cases D. 2, D. 3, and D. 4; and to Captain E. C. Lowe, N.Z.M.C., for particulars relating to cases D. 5, D. 6, and D. 7. To Captain Lowe we are also indebted for permission to publish the results of the bacteriological examinations which he made of the faeces of these patients. We have likewise to thank Dr H. S. Gettings for the results of his bacteriological examinations of the stools of cases D. 2, D. 3, and D. 7.

*Case D.* 1. Native of British Isles, who has never been abroad. Has never suffered from dysentery, chronic diarrhoea, or other intestinal complaint. Stools protozoologically examined by us repeatedly in 1916 and 1917. *Dientamoeba* first detected in January, 1917, when the case was suffering from a slight attack of diarrhoea attributed to a chill. Since then *Dientamoeba* has been especially sought at every examination, and has been found in the stools 19 times in 42 examinations. Case also infected with *Giardia* (= *Lamblia*) *intestinalis* but with no other intestinal protozoa.

*Case D.* 2.—British soldier who contracted dysentery in Salonika, 1916. Returned to England convalescent, and was discharged from hospital to dysentery depôt as bacteriologically and protozoologically negative. Whilst at the depôt the case was examined thrice by us, *Dientamoeba* being found on the last two occasions. Bacteriological examination of all three specimens negative. As patient had completely recovered clinically, he then returned to duty. No other protozoa were found in his stools.

*Case D.* 3.—British soldier, with history similar in all respects noted to case D. 2, but examined by us only twice. *Dientamoeba* found in stools only once, and no other protozoa on either occasion.

*Case D.* 4.—British soldier, who went to France in 1915. No illness in France. Went from France to Salonika, January, 1916. Entered hospital at Salonika in July, 1916, suffering from malaria and diarrhoea. Invalided to Malta, and thence to England, where he entered hospital as a case of malaria. During his stay in hospital has had three or four recurrent attacks of diarrhoea. Examined in England by us on 12 occasions, *Dientamoeba* being present in stools seven times. No other protozoa, and no *Blastocystis*, were found. Case still being treated for malaria. No records obtainable of bacteriological examinations.

*Case D.* 5.—Soldier, native of New Zealand. Has suffered ever since adolescence from "bowel troubles"—constipation alternating with diarrhoea—accompanied by abdominal pains. Treated several times in New Zealand for "inflammation of the bowels." Had an attack of "dysentery" when in tropics on troopship to England. Attack lasted a few days, and he recovered without treatment. After a short stay in England went to France in June, 1917, where he contracted acute dysentery in August, 1917. After one month in base hospital in France, entered a general hospital in England as a dysenteric convalescent. Examined by us here four times, *Dientamoeba* being found on three occasions. *Entamoeba coli* the only other protozoon found. Three bacteriological examinations made at the same time all negative. Dysentery has disappeared, but patient still suffers from his original intestinal irregularity.

*Case D.* 6.—Soldier, native of New Zealand. History closely similar to that of case D. 5, differing only in that he had no dysentery on troopship to England, and only a slight attack in France. Examined by us twice only on his return to England convalescent, *Dientamoeba* being found once. Also infected with *Entamoeba coli*, but no other protozoa found. Three bacteriological examinations in England all negative. Patient, having recovered clinically, has now been discharged.

*Case D.* 7.—Soldier, native of New Zealand. Before enlisting in 1914 had suffered from attacks of dysentery, recurring every few years, for about 20 years. Attacks severe, lasting three or four weeks, much blood and mucus being passed. Recovered on each occasion after rest in bed, without specific treatment. No causal organism ever found. Similar attacks on troopship to England, and in France, 1917. Returned to a general hospital in England, as dysenteric convalescent, September, 1917. Here examined by us seven times during September, October and November, *Dientamoeba* being found on one occasion only. Case also infected with "*Entamoeba*" *nana*. Four bacteriological examinations of stools all negative. Patient has now recovered clinically, and his stools are normal.

LONDON,
*November*, 1917.

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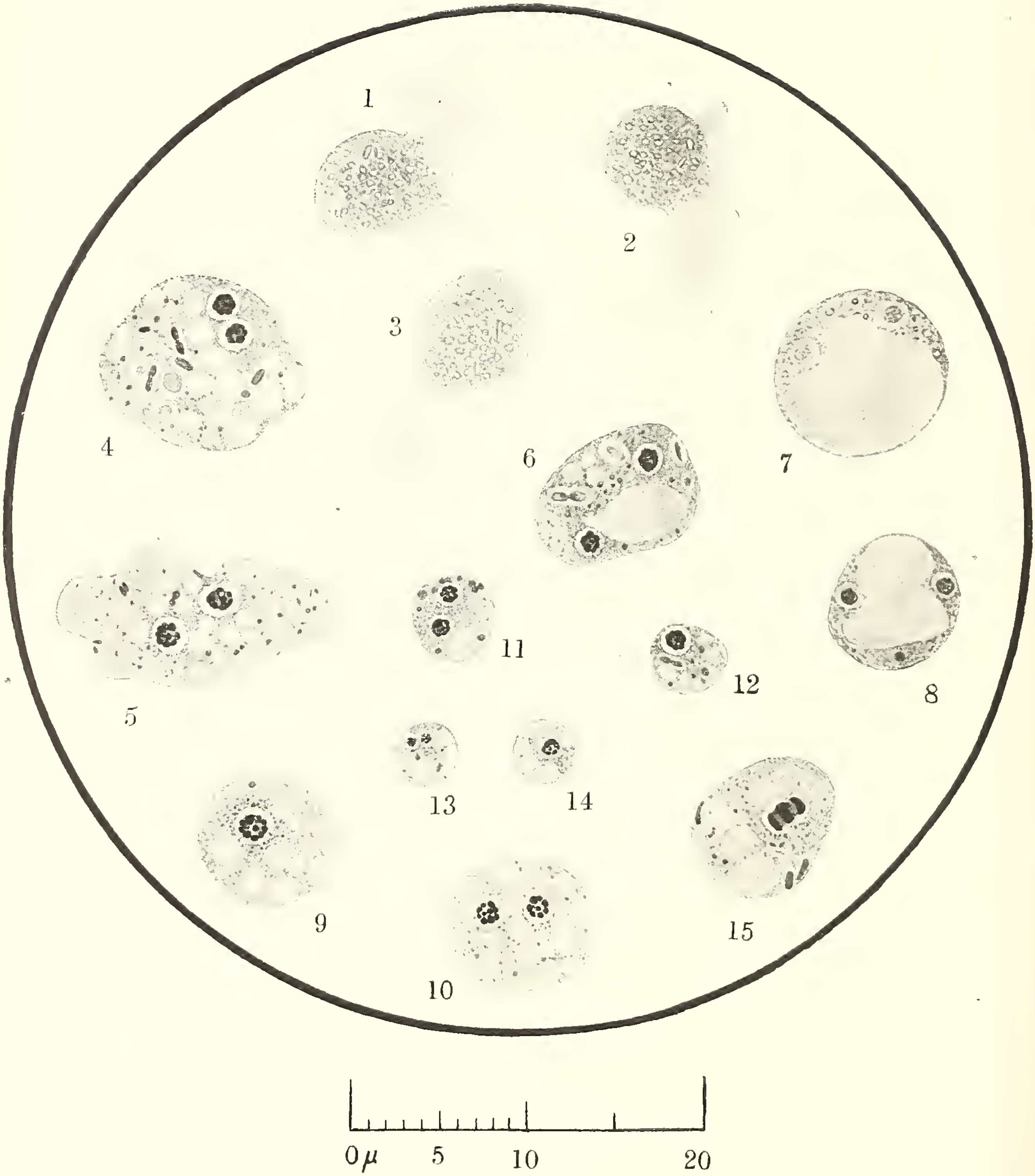
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## DESCRIPTION OF PLATE VII.

All figures depict *Dientamoeba fragilis* n. g., n. sp. The drawings of stained specimens (Figs. 4–6 and 8–15) were made with the camera lucida, and are drawn accurately to scale at a magnification of 2500 diameters. The figures of living organisms (Figs. 1–3 and 7) are drawn from freehand sketches, made approximately to the same scale. The stained specimens are from preparations fixed with Schaudinn's fluid and stained with Heidenhain's iron-haematoxylin. The drawings were made under a 2 mm. apochromat (N. A. = 1·40), with compensating oculars, and an achromatic condenser.

Figs. 1, 2, and 3. A living organism drawn at three successive moments during locomotion.
Figs. 4 and 5. Binucleate individuals of very large size.
Fig. 6. Binucleate individual of medium size, containing large spindle-shaped vacuole.
Fig. 7. Degenerated amoeba, resembling a dead *Blastocystis*. Unstained specimen.
Fig. 8. Form similar to Fig. 7, from a stained specimen.
Fig. 9. Uninucleate individual of medium size.
Fig. 10. Binucleate individual of medium size, showing structure of karyosomes when stain is strongly extracted.
Fig. 11. Small binucleate individual.
Fig. 12. Small uninucleate individual.
Fig. 13. Very small binucleate individual.
Fig. 14. Very small uninucleate individual.
Fig. 15. Individual of medium size with single nucleus in process of division.

# THE TREMATODE PARASITES OF NORTH QUEENSLAND.

## IV. PARASITES OF REPTILES AND FROGS.

By WILLIAM NICOLL, M.A., D.Sc., M.D., D.P.H.

*(From the Australian Institute of Tropical Medicine.)*

(With Plate VIII.)

The preparation of the fourth part of this paper was brought to an abrupt conclusion in November, 1915, by an illness which necessitated my return to a cooler climate. On that account the present part is incomplete, several new and interesting forms being left undescribed. These, together with the species described here, are deposited in the collection of the Australian Institute of Tropical Medicine.

Only a limited number of species of reptiles and amphibia were available for examination and there can be little doubt that a parasitic fauna of considerably greater variety will eventually be recorded.

In the first part of this paper (1914) five new species were described from reptiles and frogs, namely *Eurytrema crucifer* from the slow worm (*Delma fraseri*); *Mesocoelium microon* from the tree-frogs (*Hyla coerulea* and *H. gracilenta*) and the blue-tongued lizard (*Tiliqua scincoides*); *Dolichopera parvula* from the carpet snake (*Python variegatus*); *Aptorchis aequalis* from the freshwater turtle (*Emydura latisternum*); and the larval *Tetracotyle tiliquae* from the blue-tongued lizard (*Tiliqua scincoides*).

S. J. Johnston (1912) has described several forms from North Australian frogs and these, together with the few species mentioned in the present paper, constitute all that are known so far.

### Genus **BRACHYSACCUS** S. J. Johnston.

### **Brachysaccus juvenilis** n. sp.

(Pl. VIII, fig. 1.)

Numerous specimens of this parasite were taken from the intestine of two burrowing frogs (*Chiroleptes brevipalmatus*) in July. They were unfortunately all immature or just beginning to produce ova.

The parasite is an elongated, somewhat flattened worm, with oval outline and pointed ends. It has a distinctly yellow colour and the cuticle is smooth and unarmed.

The specimens vary in length from 1·35 to 1·65 mm. with a maximum breadth of 0·55–0·65 mm. which occurs about the middle of the body or a little behind it. The oval sucker measures 0·13–0·17 mm. and the ventral 0·19–0·21 mm., the latter being situated at a distance of 0·55–0·65 mm. from the anterior end. Both suckers are globular.

There is a distinct prepharynx in most specimens and a large pharynx measuring about 0·08 × 0·065 mm. The oesophagus is a little shorter than the pharynx, while the intestinal diverticula have a somewhat irregular course and terminate a short distance from the posterior end of the body.

The excretory vesicle is Y-shaped and consists of a long median stem which winds forwards between the testes, immediately in front of which it bifurcates into two short limbs, which do not reach the level of the ovary.

The median genital aperture is situated over the intestinal bifurcation. The cirrus-pouch is a comparatively large and conspicuous fusiform structure, extending well beyond the middle of the ventral sucker on the right side and measuring about 0·5 mm. in length. It contains a bipartite vesicula seminalis, the proximal part of which is the larger, an exceedingly long straight pars prostatica and a short ductus. The pouch is filled with a large number of prostatic cells.

The testes lie decidedly obliquely in the posterior half of the body, the anterior testis being on the left. They are separated from each other by a small space through which the excretory vesicle passes. The outline of the testes is rounded oval or somewhat cordate and their average diameter is about 0·15 mm. The post-testicular space measures about 0·33 mm. and the anterior testis is separated from the ventral sucker by a distance of 0·13 mm.

The ovary lies on the right side immediately behind the ventral sucker, which it overlaps, and the cirrus-pouch. It also overlaps the right intestinal diverticulum to a slight extent. It is a large transversely oval body, measuring 0·15 × 0·21 mm. Adjoining it there is a large oval or pear-shaped receptaculum seminis, of variable size and position. It usually lies somewhere in the space between the ovary and testes, but not infrequently it is pushed forward nearly to the ventral sucker and it may be overlapped to a considerable extent by the ovary.

The yolk glands are extensive but not very voluminous, a condition due perhaps to the immaturity of the specimens. They are entirely lateral and extend from the level of the pharynx to near the posterior end of the body. They overlap the intestinal diverticula throughout their whole extent but do not encroach upon the ovary or testes. In the post-testicular space the glands on either side remain entirely separate and they leave a considerable part of the space unoccupied. The follicles are small and scattered.

In the few specimens which contained eggs they occupied a position to the left of the ovary and the ventral sucker. There was only a small number present in any one specimen and they varied greatly in size, the limits in length being ·036–·048 mm. and in breadth ·021–·029 mm. The most normal looking eggs measured ·042–·048 × ·027 mm.

In spite of its immature condition it is obvious that this parasite differs considerably from the already known species of the genus *Brachysaccus* (*B. anartius* and *B. symmetrus* Johnston 1914). From both it differs in having the ventral sucker decidedly larger than the oral. The cirrus-pouch is much larger comparatively, while the ovary and testes are much more closely together. It is possible, however, that with the development of the uterus the testes may be pushed further apart from the ovary and from the ventral sucker and so assume a relative position more nearly approximating that in *Brachysaccus anartius*. The yolk glands are more scattered and extensive than those in *B. anartius* and differ entirely from those in *B. symmetrus*.

## Genus **APTORCHIS** Nicoll.

### **Aptorchis aequalis** Nicoll.

A further half-dozen specimens of this parasite were obtained from the intestine of a freshwater turtle (*Emydura australis*). They agree in most particulars with the single specimen already recorded from *Emydura latisternum* (see Nicoll 1914). The present specimens, however, are larger, measuring up to 3·65 mm. in length by 1·15 mm. in maximum breadth.

The oral sucker measures 0·2–0·28 mm. in diameter and the ventral 0·31–0·5 mm. The latter is situated at a distance of 0·85–1·48 mm. from the anterior end.

In an average specimen the measurements would therefore be somewhat as follows: length 2·8 mm., maximum breadth 0·87 mm., diameter of oral sucker 0·24 mm., diameter of ventral sucker 0·42 mm., distance of ventral sucker from anterior end 1·15 mm.

From these figures it appears that the species, on an average, is somewhat more elongated than the original specimen, and that the sucker ratio is about 4 : 7 instead of 4 : 6. In the original description it was stated that the intestinal diverticula terminated a short distance behind the ventral sucker. This, however, is not the case, for in a pressed preparation they can be traced, running alongside the yolk glands, to near the posterior end of the body. As they are unusually thin-walled and obscured, for the greater part of their course, by the dense folds of the uterus they are difficult to discover in total mounted preparations.

The wall of the cirrus-pouch is extremely thin and weak. The pouch contains a large bipartite vesicula seminalis which occupies about two-fifths of the length of the pouch. The remainder of the pouch is traversed by a narrow ductus surrounded by numerous cells, but no distinct pars prostatica could be detected. There is, further, no genital sinus, the vagina joining the cirrus-pouch close to the genital aperture. The appearance simulating a sinus in the original specimen was due to a kink in the terminal part of the cirrus-pouch.

## **SIGMAPERA** n. g.

### **Sigmapera cincta** n. sp.

(Plate VIII, fig. 2.)

About thirty specimens of this interesting parasite were collected from the intestine of two freshwater turtles (*Emydura latisternum*). They present several features of interest.

The species is of small size, the largest specimen only slightly exceeding 1 mm. in length. The body is flattened, delicate and transparent, while the shape is elongated with pointed ends. The posterior part is generally more expanded than the anterior part, which in some cases is greatly attenuated. The cuticle is beset with minute spines, throughout the anterior two-thirds of the body.

The specimens were of fairly uniform size, the average length of the best extended being 1·08 mm. The maximum breadth in the posterior half of the body is 0·28 mm. The globular oral sucker has a diameter of 0·066 mm., while the inconspicuous ventral sucker measures 0·07 mm. The latter lies behind the middle of the body at a distance of 0·65 mm. from the anterior end.

There is a long delicate prepharynx, with a small pharynx, followed by an oesophagus of nearly the same length as the prepharynx. The intestinal bifurcation is at a distance of 0·34 mm. from the anterior end of the body. The intestinal diverticula are long and narrow and extend to near the posterior end.

The excretory vesicle is a conspicuous and characteristic structure. It is Y-shaped with a short wide median stem and branches which diverge at a wide angle. The latter are about half as long as the main stem. The vesicle separates the testes which lie in the angles formed by the main stem and its branches.

The genital aperture is median and lies about 0·065 mm. in front of the ventral sucker. The cirrus-pouch is relatively large and has a somewhat sigmoid shape. It is stoutly built and contains a simple oval vesicula seminalis, a well-marked elongated oval pars prostatica and a short wide ductus ejaculatorius. The pouch lies over and around the left border of the ventral sucker and its total length is about 0·2 mm. The testes lie near the posterior end of the body between the excretory vesicle and the intestinal diverticula. They are symmetrically situated and of oval outline, their long axes being oblique. They measure about 0·08 × 0·05 mm.

The ovary lies a short distance behind the end of the cirrus-pouch and a little to the left side. It is a transversely oval body with a long diameter of 0·085 mm. Immediately behind it lies a small receptaculum seminis. The yolk glands are well developed and extend from the posterior end of the body forward to a considerable distance (0·07 mm.) in front of the level of the genital aperture. They are, for the most part, marginal, but in front of the genital aperture they stretch inwards and usually form a continuous band across the body. In some cases they do not quite unite. The follicles are small and rather scattered.

The uterus is short and contains only a few eggs which lie between the ovary and the genital aperture, chiefly on the right side. They measure 0·031 × 0·015–0·016 mm.

### Genus **ACANTHOCHASMUS** Looss.

#### **Acanthochasmus quaesitus** sp. inquir.

Over two hundred specimens of a species of *Acanthochasmus* were taken from the intestine of a crocodile (*Crocodilus johnstoni*) which was killed at Armadale, North Queensland. Unfortunately a considerable time had elapsed before the animal was brought for examination and by then the parasites were in a macerated condition. As a consequence the majority had lost all trace of spines but several showed remnants of the cuticular spines with a few cephalic spines.

Most of the specimens had died in an abnormally extended condition and many of them were so greatly etiolated that they presented the appearance of mere threads. On that account no attempt will be made here at a description which at best could be only conjectural and misleading. Sufficient details were available however to satisfy me that the species is distinct from any of the already known species of the genus.

### Genus **LECITHOCHIRIUM** Lühe.

### **Lecithochirium dillanei** n. sp.

(Plate VIII, fig. 3.)

A single specimen of this species was taken from the alimentary canal of an unidentified sea-snake (*Distira* sp.). It was a small, dark-red, somewhat elongated Trematode measuring about 2 mm. in length. It was somewhat flattened in preserving and the maximum breadth of the preserved specimen is 0·78 mm. The cuticle especially in the posterior part of the body was thrown into numerous irregular folds or wrinkles.

The appendix was entirely retracted within the body, appearing as a series of irregular folds. Its length in the retracted state is 0·65 mm.

The subterminal oral sucker measures 0·17 mm. The ventral sucker, which is situated at a distance of 0·7 mm. from the anterior end, measures 0·46 × 0·51 mm., so that its diameter is approximately three times that of the oral sucker.

The pharynx is contiguous with the oral sucker and measures 0·1 × 0·08 mm. There is no oesophagus; the intestinal diverticula diverge widely and passing along the sides of the body become obscure beyond the uterus.

The testes lie close together immediately behind the ventral sucker. Of irregular oval outline they measure 0·13 × 0·1 mm. The "cirrus-pouch," which lies immediately in front of the ventral sucker and extends forwards to the posterior border of the pharynx, is a weak-looking sub-globular structure with a diameter of about 0·23 mm. Its internal structure was somewhat indistinct. The external vesicula seminalis could not be seen. What appeared to be the pars prostatica was a narrow tube passing obliquely through the pouch and entering a dilated, almost globular, terminal portion, having a diameter about half that of the pouch.

The ovary lies behind and to the left side of the left testis. It is sub-globular and slightly smaller than the testes. Immediately behind it lie the digitiform yolk glands of which only five, of the usual seven, portions could be defined. They are short and irregular. The uterus, which was very poorly developed, formed a series of straggling convolutions behind the genital glands. The eggs which were of the small Hemiurid type were not measured.

The obvious defects in the foregoing description are to be accounted for by the fact that my examination of the specimen was not complete at the time of my departure from Townsville.

### Genus **DOLICHOPERA** Nicoll.

### **Dolichopera macalpini** Nicoll.

(Plate VIII, fig. 4.)

A description of this species which was obtained from the tiger-snake and copperhead snake in Victoria and New South Wales has already been published in *Parasitology* (vol. x. 2). A figure of the species is reproduced on Plate VIII.

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## EXPLANATION OF PLATE VIII.

Fig. 1. *Brachysaccus juvenilis* (× 100).
Fig. 2. *Sigmapera cincta* (× 100).
Fig. 3. *Lecithochirium dillanei* (× 50).
Fig. 4. *Dolichopera macalpini* (× 40) from *Notechis scutatus*.

The following letters apply to all the figures:

Ap. Appendix.
C.B. Cirrus-pouch.
D.St. Yolk glands.
Ex. Excretory vesicle.
J. Intestinal diverticula
Ov. Ovary.
P.G. Genital aperture.
R.S. Receptaculum seminis.
T. Testes.
Ut. Uterus.

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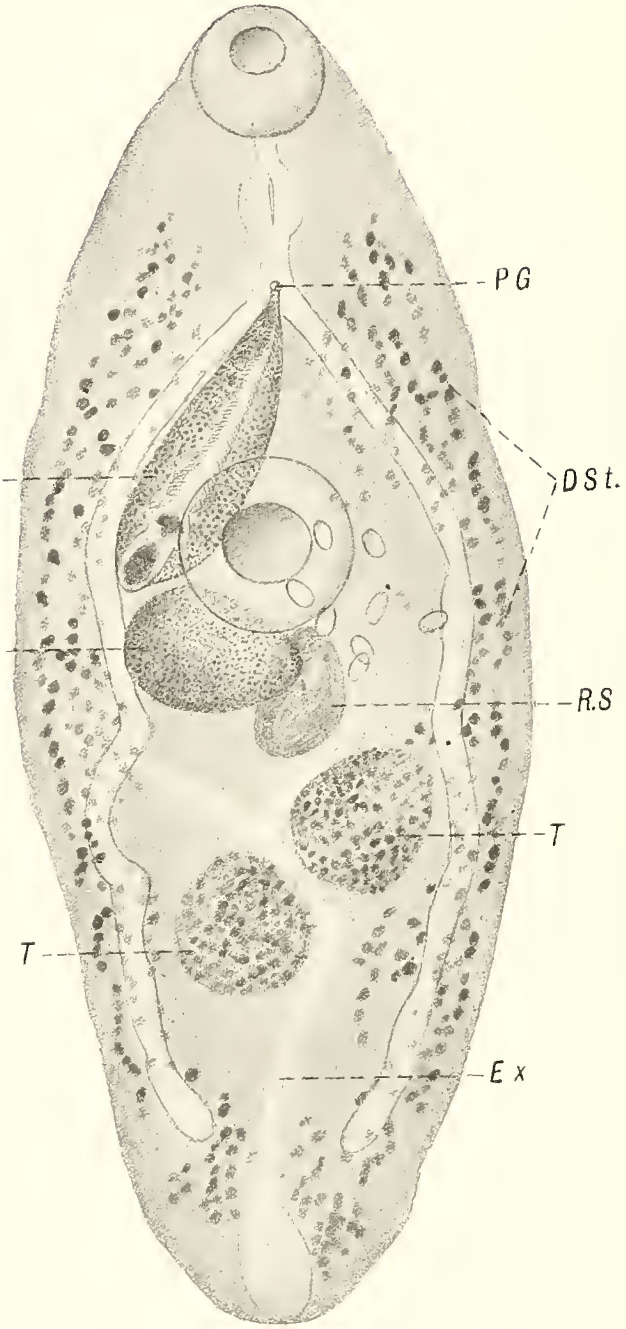

Fig. 1. *Brachysaccus juvenilis*. From *Chiroleptes*. ×100.

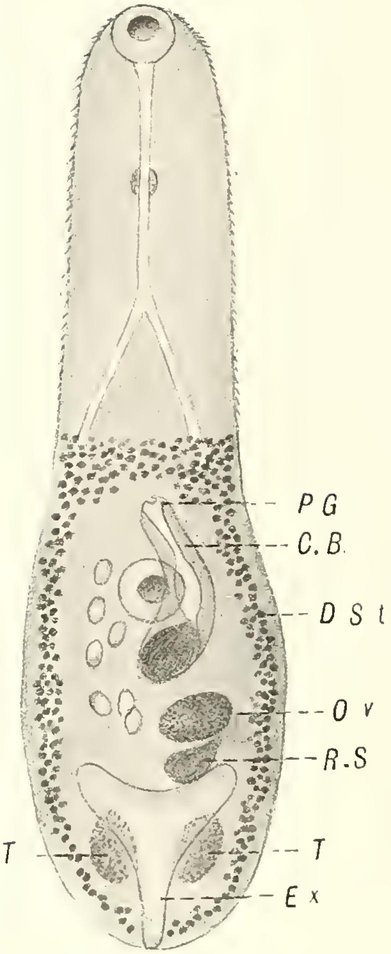

Fig. 2. *Sigmapera cincta*. From *Emydura latisternum*. ×100.

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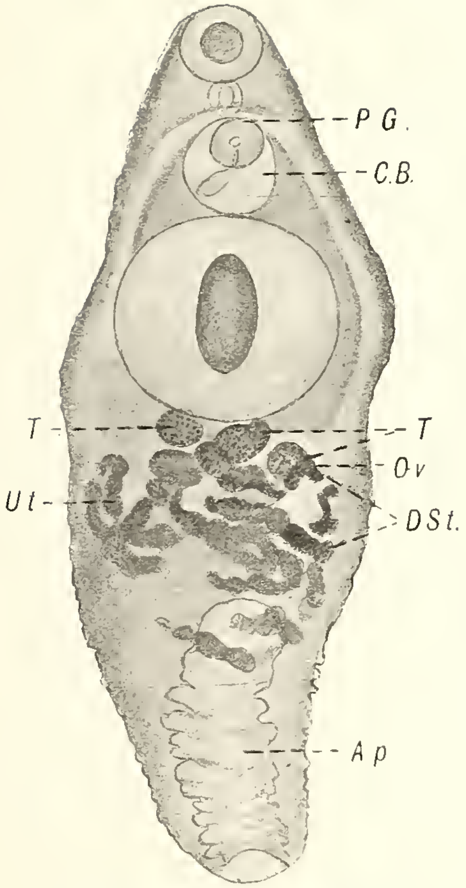

Fig. 3. *Lecithochirium dillanei.*
From *Distira.* ×50.

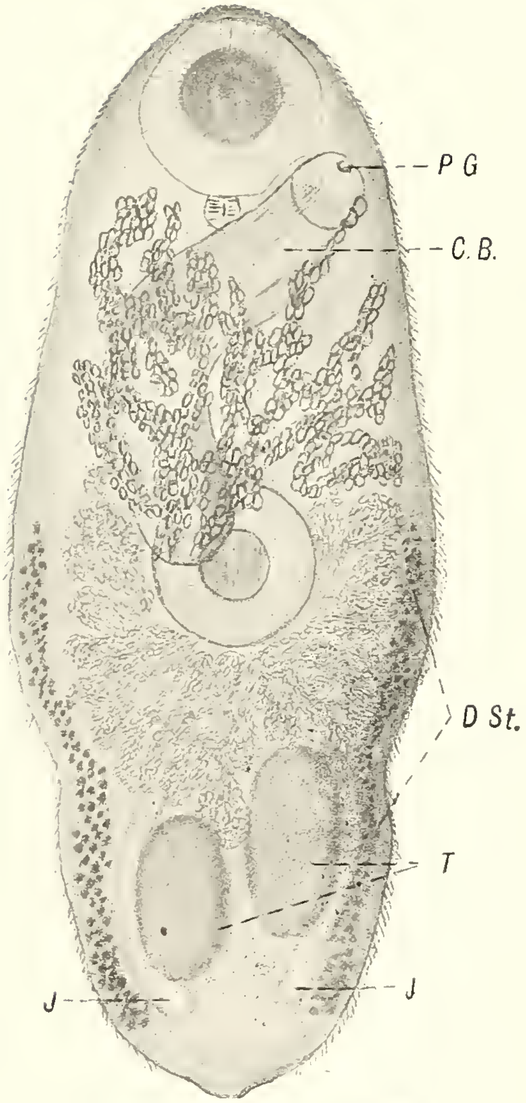

Fig. 4. *Dolichopera macalpini.*
From *Notechis scutatus.* ×40.

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# THE PATHOLOGICAL EFFECTS OF *PHTHIRUS PUBIS.*

By GEORGE H. F. NUTTALL, F.R.S.

(*From the Quick Laboratory, University of Cambridge.*)

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Compared to *Pediculus humanus*, the part played by *Phthirus pubis* in pathology is inconsiderable because: (1) it is not known to convey any infective disease, (2) it is not so prevalent, and (3) it on the whole produces slight effects, for man may be infested more frequently without knowing it.

Imhof is cited by Brumpt (1910, p. 550) as believing that the crab-louse may convey tuberculosis, but loose statements of this kind abound in medical literature and bear no weight. On the other hand, secondary skin infections may arise owing to self-inflicted scratches by infested persons in response to the itching that the parasites may cause.

That the insect is less frequently encountered on man than *P. humanus* (*capitis* and *corporis*), is exemplified by Greenough's Boston statistics (already quoted, see p. 84) wherein out of 864 verminous persons admitted to hospital, only 27 (roundly 3 %) were found infested with *Phthirus*.

## GENERAL EFFECTS OF PARASITISM.

Although Pinkus (1915, p. 239) states that crab-lice are not especially important in this war, I have had ocular evidence to the contrary in a limited way, given an instance in which a considerable number of soldiers in barracks became infested by these parasites which caused some of the men many sleepless nights.

*Pruritus* is the first symptom that draws attention to the presence of crab-lice; it can be violent and lead to much scratching day and night.

The itching may however be moderate or totally absent, but, as Payne (1890, p. 209) points out, the lack of reaction may be individual. Dubreuilh and Beille (1895, p. 135) relate that some heavily infested persons do not even show unconsciously inflicted scratches upon their skin, and Lailler, whom they cite, states that pruritus often begins only from the moment that the individual recognizes he is lousy, after which he may go on scratching himself subsequent to the removal of the parasites. Therefore, although the itching is primarily attributed to the toxic saliva of the louse it may be partly self-induced.

A remarkable case of long continued tolerance of crab-lice on the person is that described by Hewetson (1894, p. 19), to which reference will again be made, wherein an Austrian soldier intentionally remained parasitized for ten years. It is evident that the insects put the soldier to little inconvenience for he protested against their removal.

Apart from the discoloration of the skin that is commonly induced by the crab-louse and which will be presently referred to, the secondary inflammation is usually slight (Osler, 1892, p. 15), although there may be *papular eruptions* complicated by *eczematous inflammation* (Morris, 1911, p. 556). These effects, as Dubreuilh and Beille (1895, p. 135) point out, are *chiefly due to eruptions following scratching*, the papules having their tops scratched off, the eczema, etc., being localized in the pubic and axillary regions. In a soldier suffering from a heavy generalized infestation, whom I had under observation in the summer of 1915, besides numerous excoriations on the trunk and limbs, there was severe eczematous inflammation present in both axillae where hundreds of *Phthirus* were found, the hair being glued together by partly inspissated secretion, the armpits and shirt beneath being bloody from continual scratching. Where, as in Hilgenberg's (1854, vide Bibliography, p. 20) and also Fischer's case (see p. 387) crab-lice are present on the upper eye-lids, they may cause considerable irritation, whilst Dubreuilh and Beille (loc. cit) state that the lice may cause blepharitis of the ciliary borders of the lids with a variable amount of pruritus.

*Fever, headache, etc.*, attributable to no other cause according to Payne (1890, p. 209), may perhaps be due to the toxic action of *Phthirus*[1]. It is conceivable that crab-lice may cause a rise in body temperature like that Payne has seen occur after mosquito bites; a similar case has also come to my notice. Crab-lice may therefore produce effects which cannot be belittled.

---

[1] As bearing on this matter, I append a note by way of an addendum to what is stated on p. 78 regarding *P. humanus* producing fever by its bites. Jamieson (1888, p. 321) records two cases in which body-lice caused fever: (1) girl of 14 years, severe pruritus, covered with lice; temperature fell from 103° F. at once when she was freed from parasites.

### *Maculae caeruleae.*

The occurrence of *Phthirus* upon the body is usually signalized by the presence of bluish spots in the skin to which French authors have given the frequently quoted names of "taches bleuâtres" or "taches ombrées." These spots were formerly included among the rashes met with in typhoid fever and were thought to possess some relation to this disease whereas they are purely adventitious. As Dubreuilh and Beille (1895, p. 137) correctly point out, the bluish spots were doubtless formerly associated with typhoid because clinicians frequently examined the abdomen closely for the specific rose rash of typhoid, a corresponding degree of attention not being given to abdominal inspection in other affections.

*Character of the spots.* The maculae are few in number. Osler (1892, p. 15) describes them as pale blue, "sub-circular, from 4 to 10 mm. in diameter, of irregular outline and most abundant about the chest, abdomen and thighs. They sometimes give a very striking appearance to the skin. It can be readily seen that the injection is in the deeper tissues and not superficial." After Osler's attention was drawn to the association of these spots with lice, he "met with no instance in which these were not present." I may add that the spots are painless and do not disappear on pressure; more accurately speaking, their colour is pale bluish-grey, a good light being required in which to see them. The spots may at times measure 2 and even 3 cm. across, they are most noticeable in skin regions that are sparsely haired, and usually disappear in about a week after removal of the lice. It should be noted that the maculae do not occur on all persons that are infested with the louse (Moursou, Duguet, Gibier).

*Phthirus the causative agent.* That the maculae are attributable to crab-lice only was first shown by French authors. Falot (1868)[1] regarded

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(2) Healthy youth of 19 years, admitted to hospital on two occasions with a temperature of 106·2–106·4° F., much pruritus, infested with an immense number of lice; the lad was bathed and his clothing changed, after which his temperature at once fell to normal. This case was communicated to Jamieson by Dr Wood, who has seen febrile attacks due to *Pulex irritans*, cured in the same way.

[1] I have been unable to trace this reference. The date is given as 1860 by Knott (1897); Brumpt (1910) cites Falot and Moursou (1868); Dubreuilh and Beille (1895, p. 138) who cite Falot (1868) give the fullest account, and I quote from them. Gibier, Hewetson, Blanchard, and others give no reference. Simon (1881) and Mallet's (1882, vide Bibliography) papers are unfortunately inaccessible.

them as the cause, and his pupil Moursou (1877–78)[1] published the results of many clinical observations which showed that the spots were invariably associated with the presence of crab-lice, although the latter might be present without the maculae[2]. Moursou found no spots in the early stages of infestation, they only appeared 12–21 days later, i.e. when the lice had reached the adult stage (compare with my experimental observations described on p. 380 *infra*).

Duguet (1880–1–2) first *demonstrated experimentally* that the spots are due to *Phthirus*, by puncturing the skin with a lancet charged with the substance of the crushed lice, typical spots appearing at the points of inoculation after an interval of 12 hours. He next cut the lice in pieces and inoculated men with the substance obtained from the head, the forepart, middle, and hind part of the louse's body. The only part of the insect which produced the spots when inoculated was the thoracic region, corresponding to the portion whence spring the second pair of legs, i.e. the region which includes the louse's *salivary glands*. If these glands were torn out with the head, then the crushed head produced a spot when inoculated. When Duguet inoculated the substance of crushed nits, the result was negative.

Ciuffu (1907, p. 260, cited by Tièche) also produced the spots by subcutaneous inoculation, but was unable to do so by scarifying the skin.

*Cause of the coloration.* Oppenheim (1901*a*, p. 451)[3] states that *Phthirus*, by means of a ferment (oxydase) that is present in its salivary glands, is capable of forming a pigment, similar to biliverdin, which is derived from human blood and is deposited in the insect's fat body. According to Oppenheim, this pigment is likewise formed in the human body when the louse bites, the pigment giving rise to the maculae.

Oppenheim mentions that the pigment was observed by Ehrmann to occur in the cells of the insect's fat-body at the sides of the thorax and abdomen, and that it is not identical with biliverdin. In following the development of *Phthirus*, I observed that the green coloration of the louse's body in the regions indicated, appeared as the insect attained sexual maturity, being most marked in the oldest adults. The significance of this pigment remains to be determined. Oppenheim's statement that the pigment is formed by a ferment in the salivary glands, (*a*) in the louse's body, and (*b*) beneath human skin, appears to me to be an unwarranted assumption, there being no evidence whatever that the bluish spots are caused by the pigment which occurs in the louse's fat-body.

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[1] For references see Bibliography, p. 27.

[2] Brault and Montpellier (1914, p. 78) record a case of general infestation in an Arab on whose dark skin no spots were visible.

[3] I cite from the author's abstract, the full paper being inaccessible.

Huguenay (1902, *Gaz. des hôp.*, p. 591, cited by Tièche) believes that the saliva of the louse is toxic and that it alters the haemoglobin in some way about the seat of the bite. Vignolo-Lutati (1909, pp. 365–378) cites Gravagna (no reference) as attributing the pathological effects of crab-lice to a toxin, but he does not share this author's views. In other words, the authors above quoted regard the spots as due either to a genuine pigmentation (Oppenheim) or to a toxic erythema (Huguenay, Gravagna).

According to Ciuffu (*loc. cit.*) an extract of crab-lice is not haemolytic. He remarks that the maculae do not go through the series of colour changes that bruises do in fading, and therefore concludes that blood has nothing to do with the causation of the spots. He found, moreover, that the extract of *P. humanus* does not produce similar maculae. Finally Tièche (1908, p. 327) took up the problem experimentally. He cut up crab-lice in water or salt solution upon a slide, mixed the substance with blood and observed that the blood assumed a violet colour after 1–5 minutes in the vicinity of the louse fragments. He concluded that this colour change was merely due to the reduction of the oxyhaemoglobin by the fresh louse tissues, the violet colour disappearing when he admitted air by lifting the coverglass from the preparation. Spectroscopic examination confirmed him in his belief. He found that fragments of flies, *corporis*, or rabbit muscle, produced an identical effect when mixed with blood.

Like other observers, Tièche noted that the maculae disappear if the skin is irritated. *Phthirus* usually does not irritate the skin, whereas *P. humanus* does, whence he explains the absence of maculae in cases of infestation with the latter species. Tièche concludes that the maculae are probably due to the infiltration of the skin with corpuscular elements beneath the epithelial layer, the blood colour being altered.

## EXPERIMENTAL OBSERVATIONS ON THE EFFECTS OF PHTHIRUS BITES.

Tièche (*loc. cit.*) states that the first effect of the bite, observed under experimental conditions, is to produce a brown spot which turns bluish after eight hours. He saw maculae attain a diameter of 3 cm. in susceptible persons and it took twelve days for them to disappear.

Having found no other record of such experimental observations in the literature available, I carried out two experiments to which the following protocols relate:

*Exp.* 1. An adult crab-louse, the sole survivor of about 200 insects taken from a soldier the previous evening, was placed on my arm on 31. VII. 15. The louse was confined in a cell. It proceeded to feed at once, and after 25 hours, not having shifted its position, it was removed and the seat of the bite marked. The bite produced no itching, but a faint, deep-seated violet spot 2 mm. in diameter was visible, and it took a week in which to disappear.

*Exp.* 2. Some *Phthirus* eggs, taken from a soldier, were allowed to hatch in a tube carried in the inguinal space next to the body. The freshly emerged larvae were placed on my laboratory assistant's leg and confined in a stocking on 5. v. 17. The larvae shortly began to feed, remaining fixed to one hair. There was no itching or any effect observable for four days during which the larvae stayed in the same spot. On the fifth day, in the morning, the lice being still in the same position, the seat of the bite appeared faintly pink, and by the afternoon it had darkened slightly. The larvae then moulted and moved to another place, the seat of the first prolonged feed no longer being discernible after two days, although its position had been marked from the start. *As the insects attained the adult stage, the effects of further bites became more marked, typical maculae appearing* in places where the insects had anchored themselves for 12–24 hours at a time[1]. These spots disappeared in about a week. The bites at no stage produced any itchiness.

## MELANISM.

Some authors state that the pigmentation due to *Phthirus* may be generalized, the discoloration amounting almost to blackness and involving the mucous membranes and nails. Thus Le Play and Déhu (1906, p. 141) describe such a case in a woman of 60 years, but they add that she was likewise heavily infested with *P. humanus capitis* and *corporis*, therefore, it appears to me that the melanism may have been due partly or entirely to the latter. There is no doubt that melanism may arise in the course of phthiriasis as it does in pediculosis, using these terms in their strict sense. There is nothing specific about the melanism in either case. When itching, due to the presence of ecto-parasites, is maintained, it is the scratching that largely leads to the hyperaemia, pigmentation and desquamation. I regret that I have been unable to gain access to the papers by Fabre (1902, vide Bibliography) who appears to have made a special study of the melanodermia due to lice.

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[1] Vide p. 72 where it is stated that *P. h. capitis* when maturing or adult produces more lasting effects than larvae.

## SUMMARY AND CONCLUSIONS.

*Phthirus pubis* is not known to serve as a vector of any infective disease.

The pathological effects of its parasitism on man are on the whole of slight degree. Some persons are more sensitive than others to its presence; on the one hand the louse may produce no reaction by its bite or it may be tolerated upon the person for years, on the other hand it may induce pruritus of a more or less severe character, especially in parts of the body that are most infested and where the skin is most delicate, i.e. about the genitalia, the axillae, and eyelids. All grades of pruritus are observable, scratching of which the individual is unconscious may occur, or in the severest cases the scratching goes on day and night, so that by day it may be evident to spectators and aid in diagnosis. The scratching may only begin from the moment when a person realizes that he is infested.

The pale bluish-grey maculae are a specific sign of the presence of *Phthirus* upon man, but they are not always present. They are induced by the adult louse, are few in number, painless, deep seated, do not disappear on pressure, and measure 0·2–3 cm. in diameter. The maculae mark the site of the insect's bite; they appear 8–24 hours after the louse commences to feed on the spot affected. The bites of *P. humanus* do not produce maculae caeruleae. The salivary glands of *Phthirus* and *Pediculus* give correspondingly positive and negative results when inoculated subcutaneously. The maculae disappear usually within a week after the removal of the offending parasite, and they disappear more rapidly when the skin is irritated. The nature of the maculae remains to be determined, they may be due to extravasated and altered blood.

Apart from the maculae, *Phthirus*, like *P. humanus*, fleas and mosquitoes, may cause a febrile condition owing to skin irritation, although this appears to be rare; with the removal of the lice, the fever promptly subsides. Papular eruptions and eczematous inflammation may supervene upon the presence of the louse, but they appear to be largely due to the added insult of scratching the infested skin. The melanism is of non-specific origin, being due to continued scratching which leads to hyperaemia, pigmentation and desquamation. The lesions induced thereby may lead to secondary skin infection with pyogenic bacteria.

## REFERENCES.

See Bibliography, pp. 4—42, and supplementary Bibliography to follow.

# THE BIOLOGY OF *PHTHIRUS PUBIS.*

By GEORGE H. F. NUTTALL, F.R.S.

(*From the Quick Laboratory, University of Cambridge.*)

(With 9 Text-figures.)

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## INTRODUCTION.

MOST of what has been stated with regard to the main features in the biology of *Pediculus humanus* (see p. 82), likewise applies to *Phthirus pubis*. The habits of the latter species differ, however, in one particular which requires special mention, i.e. the insect is relatively immobile when upon the host, remaining attached and feeding for hours or days on one spot without removing its mouthparts from the skin into which it has bored them.

*Geographical distribution:* The available data regarding the distribution of *Phthirus* are few, and I shall be grateful to any readers who may communicate information to me on the subject. Van Beneden (1889, p. 126) is certainly wrong in stating that crab-lice have "only been found on white races," as the sequel shows:

*Europe:* Crab-lice occur apparently throughout Europe, but so far I have only seen examples from England, France and Germany. The following records relate chiefly to specimens received or examined by me. *Asia:* (N. 242) Nits taken from the eye-lashes of a Samper boy, aged 7, at Kasauli, India, 1917, by J. L. Mitter; the latter informs me that he failed to find the active stages. *Africa:* (N. 219)[1] from natives, Fort Jameson, N.E. Rhodesia, 1910, S. A. Neave; (N. 221)[1] ditto, from Zomba, Dr H. S. Stannus, and (E)[1] Blantyre, Nyasaland, 1910, Dr J. E. S. Old; (N. 220)[1] from axilla of *dog*, S.E. shore of Lake Nyasa, 1910, S. A. Neave; Mr Claude Fuller informs me that the species occurs in Natal. Mr W. M. Aders (I. 1918) writes from Zanzibar that crab-lice are rare among African negroes owing to their cleanly habits; they shave their armpits and pubes. They are found on negroes who allow their pubic hair to grow, and Mr Aders has taken them from various Indians including Parsees. *America:* (N. 74) from man, Baltimore, Md., U.S.A., 1885, G. H. F. Nuttall; (N. 227) ditto, New Orleans, La., U.S.A., 1917, M. B. Mitzmain; (N. 238) from man and *dog*, from Panama, 1917, C. B. Williams. Mjöberg (1910, p. 171) records crab-lice from Cuba. *Australia:* According to Nicoll (1917, p. 280), who was stationed at Townsville, Queensland, the species is common in Tropical Australia.

## I. PREVALENCE AND MODES OF DISSEMINATION.

### Prevalence on Man.

Reference has already been made to Greenough's statistics (p. 84) wherein ca. 3 per cent. of verminous persons admitted to hospital in Boston, Mass., were found to harbour *Phthirus*, this figure representing their relative prevalence only. I have been unable to discover any record of their incidence on a population as a whole. It has been stated that the insect is more commonly present on individuals leading an active sexual life, and this may be true, since in the opinion of those best competent to judge, in most cases, infestation takes place through coitus. Prostitutes of the lower class are considered by like authority to be the most commonly infested class. On the other hand, owing to infestation occurring from man to man, as has frequently been observed in barracks, soldiers may very frequently become infested. Lieut. A. D. Peacock, R.A.M.C. (Ms. Report, I. 1918), among soldiers selected at random, found two out of thirty men of the R.F.A., and one out of twenty-five men of the R.A.M.C. to be infested with crab-lice. The occurrence of the parasites on old people and children is relatively rare.

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[1] Received from the Imperial Bureau of Entomology.

### Occurrence on other Hosts than Man.

To my knowledge *Phthirus* has not hitherto been reported as having been found on another host than man. I have two instances to record of its presence on the dog (see under geographical distribution, p. 384). In one case ten adults were collected from the axilla of a *dog* by Mr S. A. Neave on an expedition undertaken for the Imperial Bureau of Entomology. The number of parasites present in this situation on the dog renders it evident that the insect had found a fairly suitable habitat. The second case is that supplied by Mr C. B. Williams of the Agricultural Department, Trinidad, W.I., who kindly sent me a mounted specimen that had been found on a dog at Bocas del Toro, Panama. It would be of interest to determine if *Phthirus* can be raised experimentally upon the dog.

### The Distribution of *Phthirus* on Man's Body.

The parasite is *usually confined to the pubis and peri-anal region* as common experience shows. It may, however, spread upward on the abdomen and breast and down the thighs, or it may become disseminated practically all over the body. Its spread is doubtless facilitated by scratching which may be unconscious or the reverse. Occasionally in young children, rarely in adults, the louse may be found localized upon the head, especially upon the *eyelashes*, eyebrows, more rarely on the scalp and then only along the margins of the hairy parts and occasionally in the beard. Linnaeus (1761, p. 475) states of crab-lice "Habitat in hominum immundorum pube et ciliis," and Fabricius (1775, p. 805) writes "Hospitatur in hominis immundi pube, rarius in superciliis." I append some case records culled from various sources:

#### *Cases of General infestation in adults.*

Woman, aged 23, active stages and nits present on the hair of the head about the occiput and on the temples, in the axillae and on the pubis (Waldeyer, 1900, pp. 494–9).

Woman, aged 22, many lice present on the pubis, in the axillae and on the head (Rona, *Monatsh. f. prakt. Dermatol.*, XXVI, 105). Patient in hospital, with lice on body and head (Dyer, *ibid.* p. 270). Both of these authors are cited by Waldeyer.

Arab, aged 45, lice very numerous on beard, moustache, eyelashes, eyebrows, head (a dozen being easily found). The lice appeared to be flourishing and plenty of nits were found in these situations (Brault and Montpellier, 1914, p. 78). A case of the kind is recorded apparently by Dyer (1897, p. 23).

Two cases, men in Dublin, generalized infestation including eyelashes and eyebrows, but none on the scalp (Knott, 1905, pp. 188, 189).

In 1915 I had occasion to examine several soldiers who were infested to a varying degree. One of them had active stages and nits present in hundreds all over his body excepting only his head, hands, and feet. The soldier was not unusually hairy. The lice and nits were found even on the back and sides of the trunk, etc., where the hairs were very far apart.

*Cases of infestation confined to the head.*

(*a*) Infants and young children:

Three cases, little girls, wherein the hair of the scalp was infested; in one the lice also occurred on the eyebrows. One of the girls, aged 7, bore 30 lice on the eyelashes, 10 on the eyebrows, many on the hairy margins of the scalp. All stages of development from nit to adult were found in these situations (Grassi, 1881, p. 433).

Three cases, children aged respectively 1½, 2 and 3 years, on whom nits occurred on the eyelashes only (Bleicher, 1882, p. 976).

Infant of 5 months, infested upon the eyelashes only, through its wetnurse (Trouessart, 1891).

Infant of 14 months, infested with many lice on the eyebrows, eyelashes and hair of scalp (Heisler, 1892).

Infant a few months old, the lice present in swarms on the head; had become infested through the mother. After the infested child had reposed for a short time in the same cradle with another infant, the latter's eyebrows became infested (Monier, 1892, *Rev. biol. Nord de France*, No. 6).

Boy of 5½ years, with lice upon eyelashes and head; the father found to be infested (Gallemaerts, 1893, *Journ. malad. cutan. et syph.*, p. 526).

Family consisting of a mother and five children aged 3, 5, 7, 9 and

12 years, all infested on the hairy scalp. The father was probably infested (Grindon, 1893)[1].

(*b*) Adults:

Man, aged 27, previously treated successfully for pubic infestation; the lice afterwards appeared on his eyelashes.

Man, aged 18, with lice on the eyelashes, head, and in axillae. (These two cases are described by Brault, 1906, p. 707.)

Woman, with normal hair, bore lice on the eyelashes, forehead and occiput, with maculae at the back of the neck. The active stages were found clinging very closely to the lashes of the upper lids only, the lashes being heavily charged with nits. With the exception of one nit found in the pubic region no lice or nits could be found elsewhere, but maculae were discovered on the abdomen (Fischer, 1910, pp. 115–118).

Furthermore, Railliet (1895, p. 828) cites Denny as having found crab-lice in the ears; an observation which appears to be unique as far as I can judge from the literature. Further instances of infestation of the head and eye-lashes by *Phthirus* will be found by reference to the following authors: Hooper, 1853, p. 354; Hilgenberg, 1854, p. 154; Stelwagon, 1881, p. 301; Hansell, 1883, p. 119; Ring, 1885, p. 647; Rosenmayer, 1886, p. 145; Schweinitz, 1888–9, p. 353; Jullien, 1891; Schwenk, 1891, p. 381; Schweinitz and Randall, 1891–2, p. 137; Burdin, 1892–3; Guyard, 1893–4; Marzocchi, 1908, p. 150 and 1910, p. 150. (See Bibliography.)

### *Infrequency of head infestation.*

Crab-lice occur very rarely on the head (scalp and eyelashes) in adults. Fischer (1910, pp. 115–118) cites Jullien (1892) as having observed it but once in 50,000 cases of phthiriasis in adults. The eyelashes are more commonly infested in children and infants and in one-fifth of the cases the insects also occur on the eyebrows. It is not so easy to discern the active stages upon the eyelids and they may be overlooked; a clue to the presence of *Phthirus* in this situation is afforded by the nits on the lashes making these appear dusty. Harkness (cited by Fischer) believes that the lice spread from the body to the head, and Blaschko is stated to have seen this occur. Pinkus (1915, p. 239), in a six years' practice among Berlin prostitutes, but once observed crab-lice on the scalp hair at the back of the neck; only two nits were found, the lice disappearing after two days.

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[1] The cases cited, beginning Trouessart and ending with Grindon, are abstracted from Waldeyer (1900, pp. 494–9), and Dubreuilh and Beille (1895, p. 133).

### *Why does Phthirus infest the head so rarely?*

In view of the frequency with which man harbours crab-lice it is of interest to find an explanation of why it so uncommonly infests the head. According to Waldeyer (1900, p. 496), this cannot be due to the influence of light, for the lice may occur on the eyelashes and eyebrows. It cannot be due to the odour of the secretions about the genitalia and axillae being more attractive (Fischer, 1910, p. 118), because the insects occur elsewhere on the trunk and limbs. Waldeyer advances the reasonable hypothesis that the site of parasitism is determined by the distribution of the hair on the skin, although he believes that the insect perhaps finds it easier to cling to the flat crinkled hair of the pubis and axilla than to the hair of the head which is finer, straighter, and round or oval in cross section. As Wihofe found, the number of hairs upon a square ¼ inch of scalp averages 223, whereas there are but 34 hairs upon a similar surface of pubic skin. Waldeyer's enumerations show that the number of hairs on the head is 6–10 times greater than upon the rest of the body. *Phthirus* does not run about like *Pediculus humanus*, its legs, when stretched apart, span a distance of 2 mm. and this indicates that it will best cling to hairs that are further apart than on the head. The distance between the hairs of the head is about 1 mm., whereas the body hairs occur 2 or more mm. apart. Waldeyer believes that *the distance between the hairs on the skin is the factor which governs the distribution of Phthirus upon man's body.* This also explains why the insect, when present on the head, occurs in situations where the hairs are sparsest.

From what I have observed of the behaviour of crab-lice on the body, I believe that Waldeyer's explanation is correct. I would note, however, that whereas the adults and larger larvae are usually found clinging to two hairs, each hair being grasped by the legs of one side only, the first stage larvae often cling to a single hair with the legs of both sides (see p. 401). Under such circumstances the young larva feeds best when grasping a thick hair, because it can hold it more firmly and it affords better support. I have seen a larva try in vain to feed whilst anchored to a thin hair, whereas it succeeded at once in doing so when transferred to a thick hair.

## **Mode of Dissemination and Infestation.**

Although the crab-louse is usually spread by coitus, there appear to be many exceptions to the rule. As the evidence of the occurrence of the parasite on infants indicates (see cases cited on p. 386), the insects may be innocently acquired; the infants, or a whole family of small children may become infested through a verminous mother, father, or other adult, or the parasite may be transmitted from child to child. Similarly, when persons live in close contact with one another, as soldiers do in barracks or billets, there is no question again but that infestation may arise innocently.

A certain number of insects become scattered through the frequent scratching that accompanies infestation, so it is possible for an individual to become infested through contact with clothing, bedding, the seat of the W.C., and possibly public vehicles and benches, and there is no doubt but that this occurs more often than is generally supposed. Apart from direct personal contact then, the parasite may be spread indirectly.

In the course of my experiments, I several times observed *the shedding of single hairs bearing freshly laid eggs*, and it therefore appears to me that this probably constitutes the more frequent mode of dissemination, although it has hitherto been completely overlooked. Any adult person can convince himself readily that pubic and body hair is shed under ordinary circumstances, by examining the floor of the tub in which he has bathed and from which the water has drained away. These hairs are continually being shed upon the underclothes, and they are more plentifully shed in the act of coitus or through scratching. It therefore needs little imagination to understand how the parasite can be conveyed either directly from person to person, or indirectly by means of shed nit-bearing hairs which may become entangled in the pubic or other hair of a clean individual.

Whereas the nit, and usually the first stage larva during the first day or two after hatching, is found attached to a single hair, the active stages cling as a rule to the bases of two hairs, therefore it is less likely that the older lice will become detached when the hair is shed, although it is of course possible that older larvae and adults may occasionally be set free.

Again, owing to its much greater structural adaptation to a parasitic life than is the case in *P. humanus*, the crab-louse is a very helpless creature when it is removed from the hair to which it normally clings. If placed upon cloth or any surface devoid of hair, it can make practically no progress. If a hair is brought near it under such circumstances it at once grasps it firmly. Under natural conditions it clings continuously to hair, shifting from hair to hair when disturbed, without relaxing its hold upon one hair before seizing the next. To detach the crab-louse without injury, it is necessary to slide it along the hair from base to apex, the grasp of the individual legs being comparable to that of a threaded needle whose eye is incomplete. Direct efforts to pull the insect away may result in a leg or two being torn off, the limb being left clinging to the hair. That such maiming takes place in nature is evident from specimens I have collected[1].

The accompanying illustration (Fig. 1) shows that the front legs of *Phthirus* are relatively feeble and sharp-clawed, the second and third leg-pairs being on the contrary stout, their claws terminating in a rounded knob. The difference between *Phthirus* and *Pediculus* in respect to the leg structure will be seen at a glance by comparing Figs. 1 and 2 in which I have shown the claws in black. The sharp and less incurved claws of *Pediculus* explain why it can progress readily on hairless surfaces, and the corresponding structures in *Phthirus* show why it is so helpless when removed from hair, the four hind legs being adapted solely for locomotion on hair, whilst the feeble first leg-pair play a very inconsiderable part in progression. In short, the crab-louse, as its

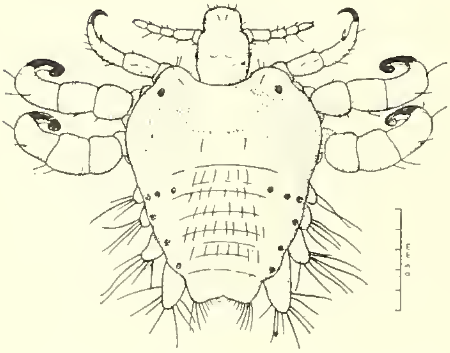

Fig. 1. *Phthirus pubis* ♀, dorsal aspect.

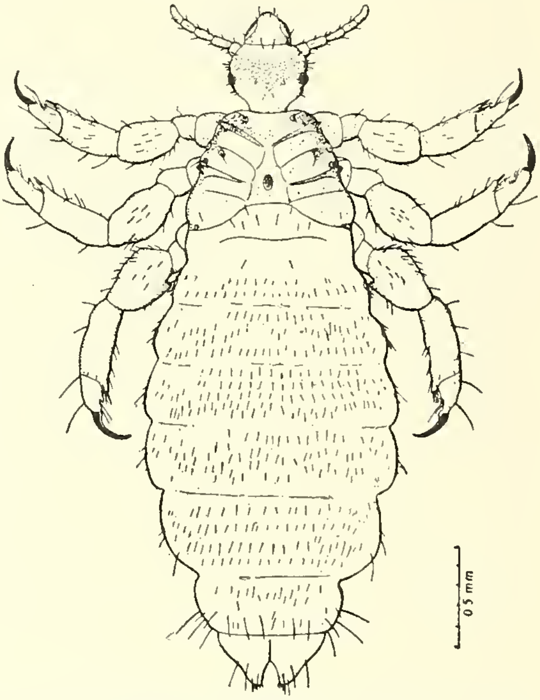

Fig. 2. *Pediculus humanus* (*corporis*) ♀, dorsal aspect.

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[1] In a maimed specimen (♀ D) that was raised experimentally, two lost legs (pairs I and II) were not regenerated during metamorphosis from the (injured) second stage larva to adult.

anatomical structure would indicate, is more prone to be disseminated passively than is *Pediculus*.

The fact that *Phthirus* in most cases remains confined to the pubic region already proves that it does not tend to wander actively. This tendency to remain localized upon a limited area of the host's skin is well exemplified in the graphic records (Figs. 7 and 8) relating to a couple of insects raised by me experimentally, neither of them in the course of their active lives wandering beyond the borders of a rectangle of skin measuring 16 × 10 cm.

## II. METHOD OF RAISING *PHTHIRUS* EXPERIMENTALLY.

Having failed to find any evidence in the literature that crab-lice have hitherto been raised experimentally, I append a brief description of the method that was employed by me successfully.

Hairs bearing single nits were removed from an infested soldier, and the nits were examined microscopically to see that they were suitable for experiment. After trying to let them hatch upon the leg by tying the nit-bearing hairs to leg-hairs, it was found more satisfactory to allow the eggs to hatch in a tube carried next to the skin in the inguinal region, the young larvae, as they emerged, being transferred to the base of a hair on the leg by means of a camel hair brush. The latter procedure was preferred because the nits were found to slip off the cut hairs when the first method was employed and the larvae often became entangled in the stocking and died without having fed. On the other hand, when the lice were carefully placed near the base of a hair so that their heads were directed toward the skin, the young larvae transferred from the tube, at once proceeded to feed and gave no further trouble. As newly emerged unfed larvae were found to die or grow feeble in a few hours after emergence, the tube in which the eggs hatched was therefore subjected to frequent inspection so that the larvae should be placed on the leg as soon as possible after hatching out.

The larvae, having been allowed to anchor themselves, the leg was covered by a closely woven black cotton stocking that was held in position by two garters placed above and below the knee. The exact spot where each insect anchored was charted by means of fixed points marked on the skin, a pair of compasses being used to register the spots on a daily record sheet. In this way the development and movements of each insect could be followed, the leg (my Laboratory Assistant's) being examined one or more times a day as occasion required, a hand lens or binocular microscope being used for observing the insects. We thus raised *Phthirus* through its life-cycle[1]. By employing a small number of insects of different ages, and observing them closely, there was no difficulty in identifying the individuals.

When the females began to oviposit, the eggs that were laid successively were marked by coloured inks and entered on the day chart, it being thereby possible in each case to determine accurately the length of time required for hatching. Occasionally the nit-bearing hairs were shed, or the larvae fell away from the skin and died upon the stocking, but, on the whole, the failures were few. As the charts (Figs. 7 and 8) show, *Phthirus* usually wanders little from the point where it first fixes itself on emerging from the egg. It stays on the same small area throughout its life. Doubtless the scratching of the skin by the host, as it occurs in nature, will tend to make the insect wander further afield, and the increasing population will thereby become somewhat scattered. There was no tendency to wander upward on the leg. The male that was raised to maturity became detached twice and gravitated downward on the leg, whence it was transferred to a spot near the females.

## III. SPECIAL BIOLOGY OF *PHTHIRUS*.

### Proportion of the Sexes on the Host.

In this connection I have but one observation to record. A total of 232 adults collected by me from a soldier, comprised 88 males and 144 females, i.e. the sexes were represented roundly in the proportions 38 per cent. male and 62 per cent. female. This is in remarkably close accord with Hindle's figures (40 per cent. male and 60 per cent. female) for *P. humanus* of which he raised 944 specimens to maturity (see p. 114). The three *Phthirus* raised by me were one male and two females. Further observations are required to establish the sex ratio, but so far the indications are that the females predominate.

### Copulation.

The process of copulation is somewhat similar to that in *Pediculus*. On several occasions, when the hairs in the vicinity of a female were touched, I observed that, whilst continuing to feed, she raised her abdomen in a manner recalling the movement of *Pediculus* females

[1] Major Howlett has since informed me that he has also raised *Phthirus* on his leg and that of his assistant in India, likewise employing a stocking.

on the approach of a male. Once, when the stocking was removed, a pair were found to have apparently been disturbed in the act, for the male was found with the forepart of his body beneath the female. The latter was clinging to two hairs and feeding in the usual way, but her abdomen was raised, whilst the male rested freely upon the skin between the hairs, with the end of his abdomen turned upward. Three days later, Dr Keilin witnessed the act and made a rough sketch of the pair in copula. From this sketch, with the aid of specimens, I have attempted to represent what was observed in the accompanying illustration (Fig. 3). The male was almost hidden by the superposed body of the female. The posterior end of the male was bent upward but the copulatory organs could not be distinguished, therefore no attempt is made to represent them in the figure. Whilst the female grasped two parallel hairs, the male grasped but one of them, he grasped a third hair with his opposite leg-pair. In neither insect did the first leg-pair appear to perform any duty. The act was completed in ten minutes.

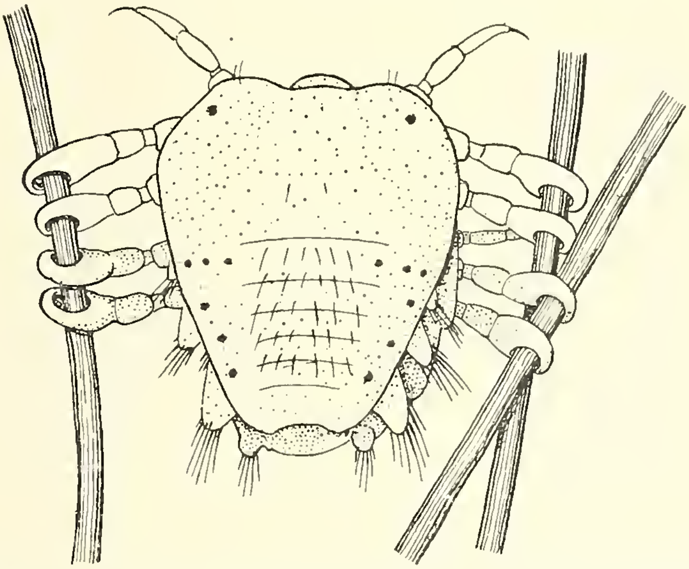

Fig. 3. *Phthirus pubis*, showing the position of the sexes when in copulation, the female being uppermost. The male grasps the hair behind the female and, contrary to *P. humanus*, does not seize her legs.

Whilst a description of the genitalia of *Phthirus* is deferred for consideration in the section on anatomy, I would note the salient feature wherein copulation differs in this species and *Pediculus* (see *Parasitology*, IX. 293). The *Phthirus* male does not grasp the female's legs, his first leg-pair are weak instead of strong and they appear to perform no function in copulation; he seizes the hair or hairs, to which the female likewise clings, with his two hind leg-pairs thereby gaining the necessary support which enables him to keep the female in position, her mouth-parts being the while fixed in the skin.

The male we raised tended to wander further afield than the two females, which suggests that he wandered in search of females; he fertilized the only two to which he had access, one of these being maimed in two legs. The latter was killed and dissected six days after ecdysis, and the neck of her flask-shaped receptaculum seminis was found packed and dilated with a mass of spermatozoa.

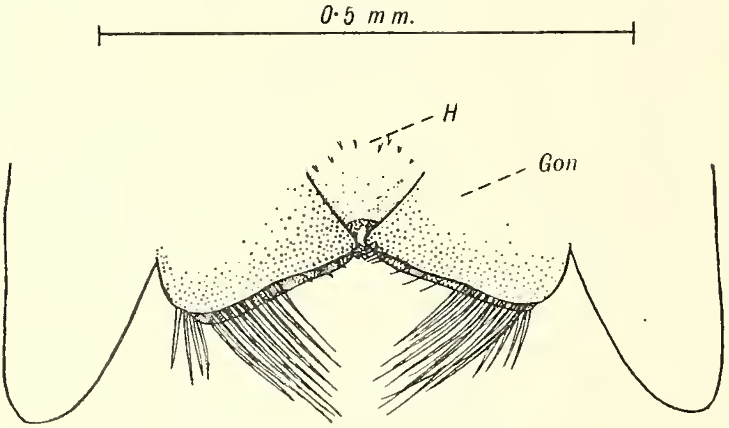

Fig. 4. *Phthirus pubis* ♀, ventral aspect of posterior end of abdomen showing (*Gon*) the gonopods converging posteriorly in the median line; slightly protruding behind them is the postero-dorsal margin of the body. Between the gonopods is (*H*) a spoon-like hollow bearing a few short spines. The hair, during oviposition, slides in the hollow between the gonopods. Compare the structures with those of *Pediculus* (p. 120, Fig. 4).

## Oviposition.

The process of egg-laying has not actually been observed by me. The female *Phthirus* (see Fig. 4) possesses gonopods of a different form to those of *Pediculus*; they consist of two short, broad-based processes bearing hairs posteriorly and they cannot surround a hair so completely as do the gonopods of the other species. These structures are figured by Landois (1864 *a*, pl. III, fig. 5, postero-ventral part of abdomen of female) but their function has not hitherto been understood. Were it not that I had demonstrated the use of the corresponding, more highly developed structures in *Pediculus*, it would be difficult to explain the purpose of the somewhat reduced organs in *Phthirus*.

Since the mode of attachment, very close to the hair-base, and the orientation of the egg are similar in both species, it is certain that the process of oviposition must be similar in essential particulars (see *Pediculus*, p. 118). The difference in the form of the cement mass that characterizes the eggs of *Phthirus* and *Pediculus* (compare Figs. 5 and 6) is, in my opinion, doubtless attributable to the form of the gonopods in the respective females, this being a subject perhaps worthy of further study. As in *Pediculus*, usually but one egg is laid upon a hair, about 0·5 mm. from its base, but the eggs may be laid in succession on one hair, a dozen or more not uncommonly being present where the infestation is heavy.

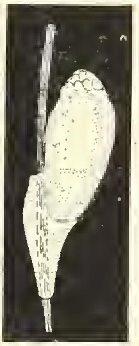

Fig. 5. *Phthirus pubis* egg on hair, lateral aspect.

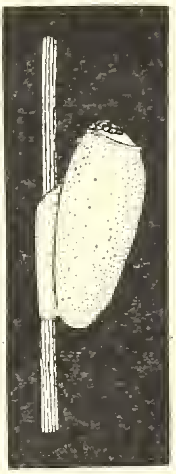

Fig. 6. *Pediculus humanus* (*corporis*) egg on hair, lateral aspect (ca. 1 mm. long; drawn to the same scale as Fig. 5).

*The number of eggs laid by a female* is stated to be 10 by Giebel (1874, p. 26), 10–15 by Blanchard (1890, p. 443) and Railliet (1895, p. 828), but there is no mention of whence these figures are derived, although they are frequently quoted by various authors. Possibly the statements are based on guesswork.

It is certain that *Phthirus* (as with *Pediculus*) lays many more eggs than has hitherto been supposed. One female that I raised laid 26 eggs, and I suspect that she died prematurely. It would not surprise me to learn that a female may lay 50 eggs or more, but further experiments are required to determine what is the full complement. The maximum number of eggs laid by a female per day was three.

## Hatching.

The period required for the hatching of the eggs is given as 6–7 days by Blanchard and Railliet (*loc. cit.*), as 6 days by Brumpt (1910, p. 550).

Of nine eggs laid on my Assistant's leg, beginning 25. v. 1917, and of which accurate records were kept, six hatched in 7 days and three in 8 days. Of ten eggs of undetermined age removed from a patient and carried in a tube against the skin of the groin, five emerged in 7–8 days, none subsequently.

### *Influence of temperature on hatching.*

Very poor results attended my efforts to hatch out eggs in the thermostat at 30–32° C., owing to there being a large percentage of deaths, even when the air was kept damp. Marzocchi (1913, p. 314), who observed hatching under these conditions, adds that he obtained negative results at 18–20° C. The influence of temperature of *Phthirus* is therefore in accord with what has been stated for *Pediculus* (see p. 143).

### *Developmental changes in the egg and mode of emergence.*

These are essentially the same as in *Pediculus* (*q.v.*); the much less transparent egg-shell in *Phthirus*, however, considerably impedes direct observation.

## Development from Larva to Adult.

Fifteen days is stated to be the period required according to authors (Blanchard, Railliet, Brumpt, *loc. cit.*) but none mention the number of moults that occur.

Timed from the hatching of the first stage larva (Day 1) my observations gave the following results:

| | Moult occurred over-night between days | | | |
|---|---|---|---|---|
| | ♂ | ♀ | ♀ | |
| 1st moult | 5–6 | 5–6 | 5–6 | 2nd stage larva emerged |
| 2nd ,, | 9–10 | 11–12 | 11–12 | 3rd ,, ,, ,, |
| 3rd ,, | 13–14 | 16–17 | 15–16 | Adult emerged. |

It is noticeable that the last two moults took place earlier in the male than in the females. The males in *Phthirus* are usually smaller than the females.

The process of *moulting* is similar to that in *Pediculus*, the old skin showing corresponding lines of cleavage. The cast skin remains clinging to the hair in the same manner as the living insect.

Record relating to the development of a male and female *Phthirus* from the egg to the adult stage, and ending with their death.

| 5. v. 1917<br>Day | MALE (see Fig. 7) | 7. v. 1917<br>Day | FEMALE (see Fig. 8) | No. of eggs laid | |
|---|---|---|---|---|---|
| 1. | Emerged as *1st stage larva* before 8.30 a.m. Placed at base of single hair at 10.30 a.m. and started to feed at once. | | | | |
| 2. | Had not moved, body swelling. | | | | |
| 3. | ,, ,, ,, | 1. | Emerged as *1st stage larva* at 11 a.m. 5 p.m., anchored on one hair and fed at once. | | |
| 4. | ,, ,, ,, | 2. | Had not moved, body swelling. | | |
| 5. | ,, ,, appeared somewhat shrivelled. | 3. | ,, ,, ,, | | |
| 6. | Moulted to *2nd stage larva*, shifted 15 mm. Now and henceforth grasping two hairs with its hind leg-pair. | 4. | ,, ,, ,, | | |
| 7. | Shifted 45 mm. | 5. | ,, ,, ,, | | |
| 8. | Had not moved. | 6. | Moulted to *2nd stage larva*, shifted 29 mm. | | |
| 9. | Shifted 14 mm. | 7. | Had not moved. | | |
| 10. | Moulted to *3rd stage larva*, shifted 4 mm., wandering. | 8. | ,, ,, | | |
| 11. | Shifted 9 mm. | 9. | Shifted 20 mm. | | |
| 12. | ,, 5 ,, | 10. | Had not moved. | | |
| 13. | ,, 6 ,, | 11. | ,, ,, | | |
| 14. | Moulted to *adult*, shifted 9 mm. | 12. | Moulted to *3rd stage larva*, shifted 11 mm. | | |
| 15. | Had not moved at 8 a.m., but by 11.30 a.m. had shifted 50 mm. up leg. Appears well fed, body dark, ca. 1·2 mm. long, claws of hind leg-pair 2 mm. apart. | 13. | Shifted 3 mm., body flat, ca. 0·75 mm. long. | | |
| 16. | Had not moved. | 14. | Had not moved. | | |
| 17. | Shifted 100 mm., wandering down leg on stocking, transferred up to near ♀ B. | 15. | ,, ,, | | |
| 18. | Had not moved. | 16. | ,, ,, | | |
| 19. | Shifted 34 mm. Copulated 19–20th day? with ♀ B. | 17. | Moulted to *adult*, shifted 40 mm., ca. 1·5 mm. long. | | |
| 20. | ,, 51 ,, | 18. | Shifted 10 mm. | 0 | |
| 21. | ,, 8 ,, [1st fertile egg laid by ♀ B.] | 19. | ,, 10 ,, | 1 | |
| 22. | ,, 15 ,, [1st egg laid by ♀ D is infertile.] | 20. | ,, 40 ,, | 2 | |
| 23. | ,, 26 ,, | 21. | ,, 3 ,, | 1 | |
| 24. | ,, 40 ,, Found wandering, put near ♀ D. Sides of male's body greenish | 22. | ,, 20 ,, | 2 | |
| 25. | ,, 9 ,, | 23. | ,, 33 ,, | 3 | |
| 26. | ,, 8 ,, Beneath ♀ D. copulated? | 24. | ,, 59 ,, | 2 | |
| 27. | ,, 29 ,, | 25. | ,, 13 ,, | 3 | |
| 28. | ,, } 35 ,, Copulated with ♀ B. | 26. | ,, 53 ,, | 2 | |
| 29. | ,, | 27. | ,, 89 ,, | 3 | Sides of ♀ B's body greenish. |
| 30. | ,, 5 ,, | 28. | ,, 22 ,, | 2 | |
| 31. | ,, 10 ,, | 29. | ,, 15 ,, | 1 | |
| 32. | ,, 3 ,, | 30. | ,, 17 ,, | 2 | |
| 33. | ,, 4 ,, | 31. | Had not moved | 2 | |
| 34. | Had not moved. | 32. | ,, ,, | 0 | |
| 35. | ,, ,, Found dead; had lived 22 days as adult. | 33. | Found dead; had lived 17 days as adult | 26 | Total eggs laid by ♀ B. |

N.B. The movements having been recorded but once in 24 hours, the chart naturally does not represent all the changes of position that took place. When it is stated that the insect "shifted" a certain distance, the measurement is given arbitrarily in a straight line from the last point of attachment to the new, irrespective of the wandering that took place from point to point. That such meanderings took place is evident from the female's chart, wherein the position of the eggs laid on hairs in a hypothetical order is denoted by clear circles as opposed to black spots denoting anchorages. (See charts, next page.)

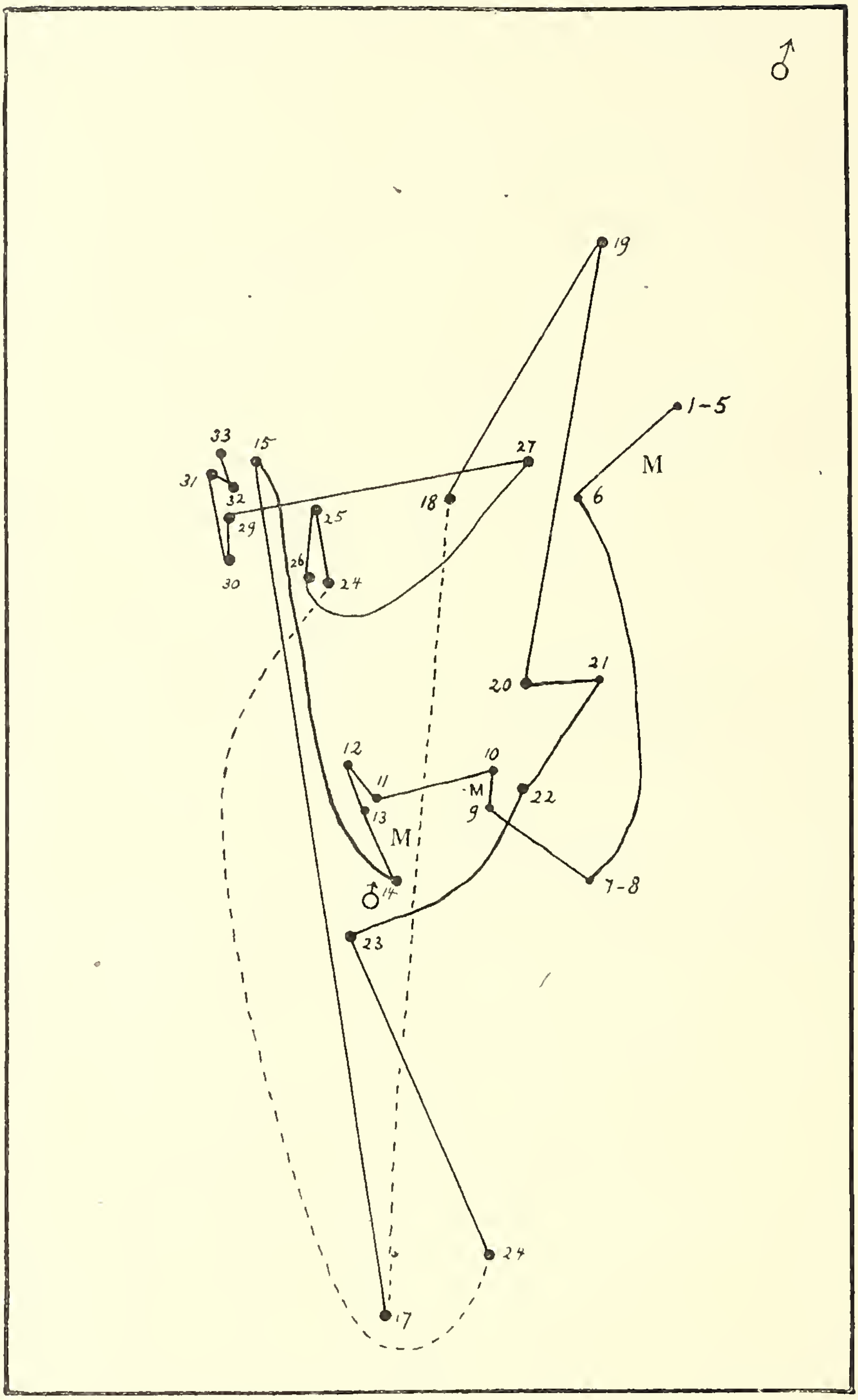

Fig. 7. Chart showing the positions occupied on successive days by a *Phthirus* ♂, from the day it hatched out and anchored itself as a first stage larva (Day 1) until it died (Day 33). The black spots denote the anchorages, M the three successive moults ending in ♂ where it became adult. The dotted line shows where the male was transferred higher up the leg. The quadrangular contour indicates the actual area of skin in which the movements took place (natural size). The movements of the female in the identical area are recorded in Fig. 8.

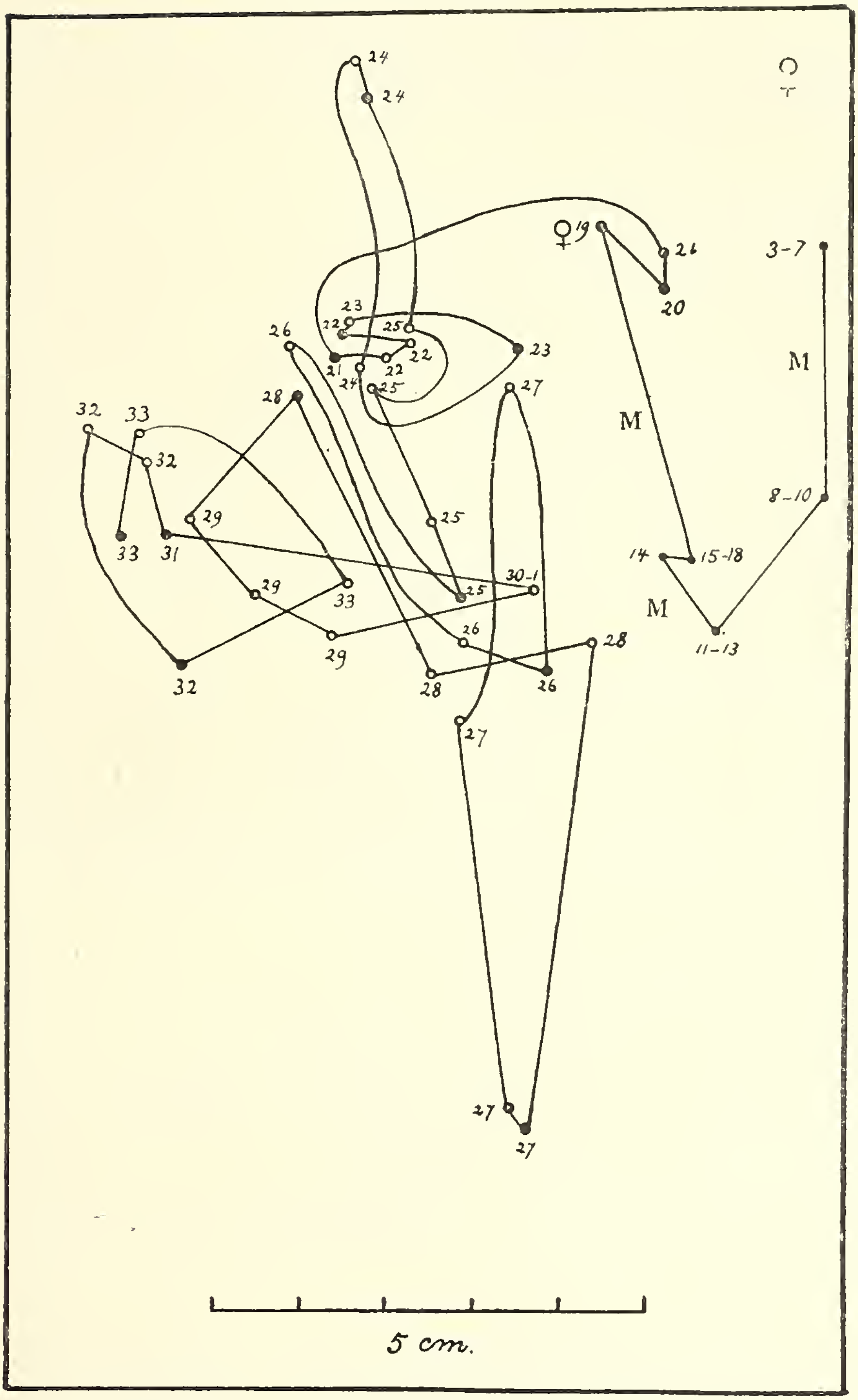

Fig. 8. Chart recording the movements of a *Phthirus* ♀ in the same way as described for the male in Fig. 7. To make the day-numbers synchronize, the female having hatched two days after the male, her day of emergence as a first stage larva is charted as Day 3 (instead of Day 1), and the day of her death is given as Day 33 (instead of Day 31). The numbers therefore correspond in point of date in both charts and the relative positions of the sexes can be compared on any given date. The *open circles* (°) along the track of the female denote where *eggs* were laid at the bases of hairs.

## Duration of the Life-cycle.

Judging from my two reared females, the life-cycle is completed in 22–27 days made up as follows:

| | |
|---|---|
| Hatching takes | 6– 8 days |
| Development to adult | 15–17 ,, |
| Pre-oviposition period | 1– 2 ,, |

Life-cycle completed in 22–27 days = Egg to egg period.

This affords no support to the assertion of Piaget (1880, p. 630) that *Phthirus* "seem to reproduce more rapidly than pediculi," on the contrary, in *Pediculus humanus* (*corporis* and *capitis*) raised by me on the body, the cycle was completed in 16–17 days (see p. 161). It is conceivable, however, that the life-cycle of *Phthirus* may be completed somewhat more rapidly on the pubis than on the leg.

## Longevity.

(*a*) Unfed, in vitro.

On dampened pubic hair placed in a corked bottle that was carried in the pocket near the body, Marzocchi (1913, p. 314) found that the insects mostly died in 10–12 hours. They died more rapidly in open vessels exposed to light. Although Galli-Valerio (1913, p. 501) states that *Phthirus* died in two days at 20° C., it appears to me probable that they died sooner than he records.

The following experiments were made by me with adults and large larvae that were kept in the dark.

| Lot collected on | No. of lice tested | Conditions in vitro | Result |
|---|---|---|---|
| 1. viii. 15, 1 p.m. | 20 | 16° C. moist | 3/20 alive after 20 hrs. |
| ,, ,, | 20 | 16° C. dry in room | all dead ,, 20 ,, |
| 2. viii. 15, 3–4 p.m. | 200 | 30° C. moist | ,, ,, 20 ,, |
| ,, ,, | hundreds | 20° C. dry in room, damp weather | 2 dead after 17 hrs.<br>8 alive after 26½ hrs.<br>all dead ,, 42½ ,, |
| | | 20° C. moist | many feeble in 17 hrs.<br>1 moving after 26½ ,,<br>all dead ,, 42½ ,, |
| | | 20° C. very moist | 2 dead after 17 hrs.<br>1 moving after 26½ hrs.<br>all dead ,, 42½ ,, |
| 3. viii. 15. 4 p.m. | many | 20° C. dry | ,, ,, 19½ ,, |

(*b*) When fed.

Of the two specimens I raised experimentally, the male lived 22 days, and the female 17 days, as adults. The insects will doubtless live longer.

### Feeding Habits.

When a newly emerged larva is placed upon a hair, close to its base, it immediately punctures the skin and proceeds to feed. It differs in its feeding habits from *Pediculus*, for it remains for days upon the same spot without appearing to withdraw its mouthparts, whilst a microscopic examination shows that it pumps blood intermittently at frequent intervals. On one occasion only did I find an insect with its mouthparts withdrawn, but they were soon reintroduced. During the first day, or for two or more days, the young larva as a rule grasps but a single hair with its four strong posterior legs (see Fig. 9), whilst its body gradually

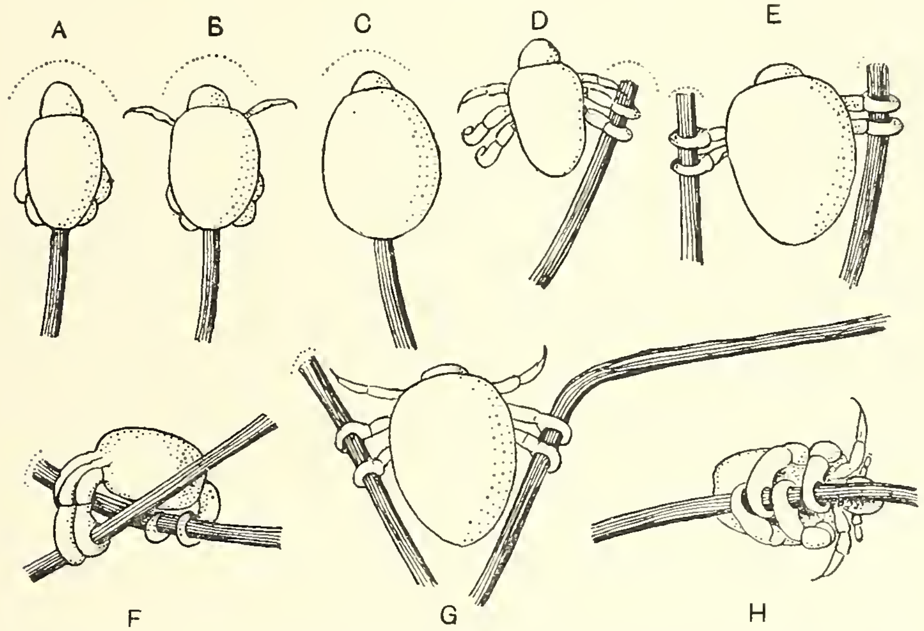

Fig. 9. *Phthirus pubis*, larvae of the first stage. A–D grasping a single hair, E, F, G, grasping two hairs; all of them drawn from life in the act of feeding. H, ventral aspect of larva killed by immersion in alcohol and mounted in balsam; it shows the manner of grasping a hair. A and B represent the same larva when respectively one and two days old, C another larva three days old, E and G others four days old, D is one day old, and F two days old grasping crossed hairs. G shows how a hair, whose root is at some distance, is bent by the insect's grasp. E shows the usual position in all stages when grasping two hairs. The arched dotted line indicates the border of the hair-pit in some instances.

swells. After this it may extend its legs so that it comes to grasp two hairs with the legs of each side as the later stages almost invariably do. The larva moults in situ and then shifts its position a short distance, and immediately resumes feeding. The following stages behave similarly. It is relatively uncommon to find the insects wandering excepting immediately after a moult, or after they attain sexual maturity, and even then they are usually found feeding. Gorging, as observed in *Pediculus*, is never seen in the crab-louse. Two tusk-like, retrograde processes, situate antero-laterally and ventrally upon the head, serve as an additional means of fixation of the mouth-parts to the skin, such processes being absent in *Pediculus* which feeds rapidly.

As an example of the manner of feeding, the following record may be cited. It relates to a hungry adult which I placed on my arm and observed microscopically. Its pharyngeal pump was seen to act and blood appeared in the gut diverticula 8′ after the mouth-parts had penetrated; after 11′ a blood droplet issued from the anus. The record proceeds: 18½′ stopped pumping, 21′ pumped, 23′ stopped pumping, 24′ inspissated blood issued from the anus and pumping recommenced, 25′ pumping stopped and five beads of blood were voided, 27′ started pumping, 29′ stopped, 29½′ started again and voided a drop of blood, 30½′ stopped, 37′ started, 38′ stopped pumping, and so on. The next day the same kind of observation was repeated, and the examination of other insects subsequently showed that this is the ordinary mode of feeding, pumping and resting periods alternating and blood-red faecal matter being voided at short intervals. This frequent defaecation renders their surroundings very filthy in a short time. Their filthiness in this respect is much more observable than in *Pediculus* because defaecation is so localized. Merely because the faeces are more scattered, *P. humanus capitis*, relatively speaking, appears to produce less evident filth, whilst *corporis*, favoured by its retreats in the clothing, ranks third in this respect.

The habit of what practically constitutes continuous feeding which characterizes *Phthirus*, sufficiently explains why it dies so quickly from hunger and drought when removed from the host.

## Reactions to Light and Heat.

On short exposure to light, when feeding, *Phthirus* frequently shows signs of restlessness, but it does not release its hold and wander away. As Howlett (1917, p. 188) showed, the insects are little affected when a tube containing hot water is brought near them whilst feeding, but otherwise they "become greatly excited and move with remarkable eagerness and rapidity," and if placed on their backs on a table and a hot tube approaches them, their "wild efforts to reach it attain an intensity which is almost pathetic."

Although the pubic and peri-anal and axillary regions are the sites of selection for its parasitism, there is no evidence that these habitats are chosen because of their higher temperature and moisture. The selection of these regions seems to depend only upon the character of their hairiness.

## SUMMARY AND CONCLUSIONS.

Although *Phthirus pubis* occurs in Europe, Asia, Africa, North America and Australia, and is found on negroes as well as whites, as herein recorded, further data are required relating to the geographical distribution of the species and the races of man it infests.

The crab-louse occurs less frequently on man than does *Pediculus* and appears to be parasitic chiefly on persons leading an active sexual life. It has, so far, only been twice recorded on another host than man, i.e. the dog.

Whilst the crab-louse is usually found confined to the pubic and peri-anal region, it frequently spreads upward upon the abdomen and breast, and may infest the axillae severely, or it may spread downward along the thighs. Generalized infestation is rare, in such cases only the hands and feet are spared, although the head and neck are as a rule also free from parasites. The crab-louse has occasionally been found localized upon the head in infants, being either confined entirely to the eyelids, the nits being encountered on the eyelashes, or occurring also upon the eyebrows and hairy portions of the scalp. In adults, the parasite occurs much more rarely in these situations; a case is recorded of an Arab in whom, in addition, the beard and moustache were infested. Such cases are rare in children, very rare indeed in adults.

Apparently the head is so rarely infested because it is less suitable as a habitat. As Waldeyer points out, the head is probably not a suitable habitat because the scalp-hairs are crowded close together and finer than on the pubis and in the axillae. The majority of the active stages are found clinging to two hairs on the regions of the body they most infest, and where the hairs are sparser than on the head. In these parts, the hairs are 2 mm. or more apart. The reach between the extended two hind leg-pairs of the adult insect is about 2 mm., these legs being the ones that are used for grasping the hair.

Whilst infestation usually takes place through coitus, there are many exceptions to the rule as proved by the occurrence of crab-lice on infants. The latter may become infested by their parents or other adults. The insect may pass from one infant to another through their sharing a cradle, or it may pass from soldier to soldier when crowding occurs in barracks or billets. Scratching of their persons by infested individuals no doubt aids in the spread of the parasite upon them and the dissemination of the insect further afield. Both the act of coitus and scratching promote the shedding of hairs, and these, when bearing nits or young larvae, may be of considerable importance in disseminating the parasite. Such hairs, and no doubt occasionally active stages of the louse, are shed on clothing, bedding, the seat of the privy, etc., and readily become entangled with the pubic or other hair of clean persons who may come in contact therewith. A detached louse promptly clings to any hair with which it comes in contact. Therefore, whilst *Phthirus* is commonly conveyed directly, it may also be acquired indirectly. It is a helpless creature when removed from the hair to which it clings continuously upon the body, where it moves about by shifting from hair to hair; it is therefore much more likely to be conveyed passively from host to host than is *Pediculus*.

Unless disturbed, the parasite remains confined throughout its life to a limited area upon the host's skin as I have demonstrated experimentally.

The females appear to preponderate over the males in number when upon the host, their relative proportion being about 3 : 2. In copulation, the male does not seize the female as in *Pediculus*; he seizes the hairs to which the female clings, using these as a support. Oviposition occurs as in *Pediculus*. A female that was raised experimentally, laid up to three eggs per day, laying a total of 26 eggs. The hatching period (on the leg) lasted 7–8 days. Like *Pediculus*, the crab-louse passes through three moults (there being as many larval stages) before it attains sexual maturity. When the adults are about 10 days old they exhibit to a well-marked degree the greenish coloration of the fat-body which is seen by transparency at the sides of the abdomen in the living insect. The life-cycle, from egg to egg, is completed in 22–27 days (on the leg).

Young unfed larvae usually die within ten hours of emergence. When removed from man, they survive longer at 16–20° C. than at 30° C. and die much more rapidly in a dry than in a moist atmosphere;

none of the numerous lice of all stages that were tested were found to survive up to 42½ hours, when maintained under different conditions. Living on man a male survived 22 days, a female 17 days, but the insects can doubtless live longer.

The feeding habits of *Phthirus* recall those of Ixodidae in being practically continuous, and, like *Boophilus*, they stop feeding to moult upon the host. After moulting they promptly proceed to feed again, shifting but slightly to a fresh feeding ground. On emerging, the young larva clasps a single hair and feeds at its base, and after a day or more it may clasp two hairs as the later stages do usually. The habit of continuous feeding explains why the insects die so quickly when removed from the host.

Whilst a brief exposure to light renders the insects restless but does not cause them to wander away whilst feeding, a warm body brought within their sphere promptly produces great activity in *Phthirus* when it is removed from man.

## REFERENCES.

See Bibliography, pp. 1 *et seq.*, and supplementary Bibliography to follow.

## CORRECTIONS.

p. 44, lines 1–3. *Delete* the passages ", as the investigations......latter disease," and *substitute* "Typhus." [The investigations of Sergent on relapsing fever did not inspire Nicolle's researches on typhus. The deleted passage was written by me under a misapprehension.]

p. 173, lines 4–5. *Delete* ", possibly those of Bacot,"

# ON THE OCCURRENCE OF A CEPHALINE GREGARINE, *LEIDYANA TINEI* N. SP., IN LEPIDOPTEROUS LARVAE.

By D. KEILIN, Sc.D.,

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(*From the Quick Laboratory, University of Cambridge.*)

(With 1 Text-figure and Plate IX.)

The gregarine herein described lives in the larvae of the moth *Endrosis fenestrella* Stain. which I found in the nests of the house-martin (*Chelidon urbica* Linn.)[1].

The gregarines are usually present in almost all the moth larvae and they are always located in the mid-gut, being there mixed with the particles of feathers, chitinous remains of other tineid larvae and grains of sand. These latter make the preparation of sections very difficult and I had to study the gregarine chiefly on living specimens or in stained smears.

A larva of *E. fenestrella* Stain. often contains a great number of parasites in different stages of their life-cycle (Text-fig. 1). In habit this gregarine is always solitary. It moves very slowly; its body is easily curved and frequently shows transverse folds (Plate IX, figs. 8, 9, 10).

The *youngest stages* of the parasite which were found are the small trophozoites about 30$\mu$ long and 10$\mu$ wide; these had their epimerit buried in the epithelial cell of the host (Plate IX, figs. 1, 2). At this stage the boundary between the protomerit and deutomerit is not yet well pronounced; the protoplasm is transparent and the nucleus, which is still visible in the living parasite, contains a big karyosome. The protoplasm of the protomerit is less dense than that of the deutomerit and it contains some highly refractive corpuscles.

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[1] The larvae of some other moths (*Oecophora pseudospretella* Stain. and *Tinea pallescentella* Stain.) found in the nests of *Fringilla coelebs* L. and *Parus ater* L. and *Hirundo rustica* L. (Cambridge and Tonbridge) also contained some gregarines very similar to that here described, but I could not discover their complete life-cycles nor find their cysts. It is therefore impossible to identify these gregarines.

I am very much indebted to Lieut. P. A. Buxton and Mrs P. A. Buxton for kindly sending me from Tonbridge the nests of different birds with valuable information about them. The material collected from these nests will be utilised for further work.

The trophozoite, while still attached to the epithelial cell of the host, increases much in size till it reaches about 130–200$\mu$ in length (Pl. IX, figs. 3, 4, 5 and 6), then it separates from the epithelium, loosing the epimerit and is liberated in the lumen of intestine becoming a free living sporont.

*The full grown sporont* is elongated, its maximum length is 300$\mu$ and maximum width 85$\mu$ (Pl. IX, fig. 7); the protomerit is hemispherical. The protoplasm of the whole gregarine is very dense and dark and the nucleus is not visible when the parasite is alive.

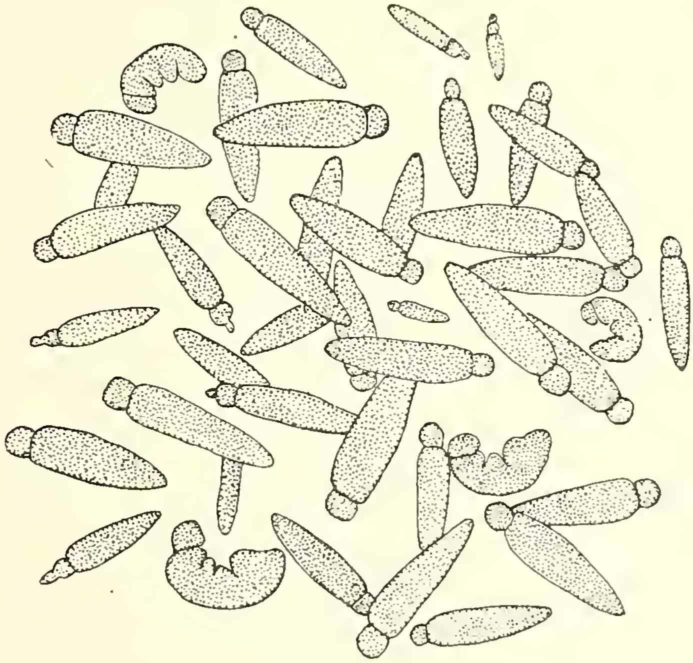

Fig. 1.

In forming *the cyst* two of these sporonts come together, simulating an association; they then fold over one against the other, uniting closely, and finally they become a spherical mass of a uniform grey colour.

These early stages of the cyst formation are usually found in the intestine of the host (Pl. IX, fig. 11). The further stages were found in the excrement of the larvae which I kept for many days (12–20) on moist blotting paper in a small wooden pill-box.

The wall of the cyst is about 8–10$\mu$ thick and is composed of many concentric layers of a highly refractive material (Pl. IX, figs. 13, 14 *c.w.*). It is also surrounded by a thick outer layer of a transparent substance (Pl. IX, fig. 13 *ex.l.*). Dehiscence is produced by means of 2 to 5 short sporoducts (Pl. IX, fig. 14 *sp.*). The spores are barrel-shaped and are 7$\mu$ long (Pl. IX, fig. 12).

The infestation of the host is produced by ingestion of the spores and it is quite possible that the non-infected larvae may become infected after eating one of their own kind which contains the parasites. Cannibalism as a cause of heavy infection of *Gryllus* by *Gregarina kingi* Crawley was mentioned by Crawley (1907, p. 223).

### SYSTEMATIC POSITION.

The following are the main characters of this gregarine: (*a*) sporont solitary; (*b*) epimerit subspherical, simple; (*c*) dehiscence by sporoducts; (*d*) spores barrel-shaped. These are quite sufficient to put this gregarine in the genus *Leidyana* Watson (1915) of the family of *Gregarinidae*. This genus has hitherto contained but two species:

1. *Leidyana erratica* (Crawley) Watson is a parasite of *Gryllus abbreviatus* Serv. and of *G. pennsylvanicus* Burm. Crawley (1903) at first considered this species to be identical with Leidy's *Gregarina achaeta abbreviata*[1]. In 1907 he found that it differs from the latter species, and described it as a new one; but he did not find its cysts and put this species in the genus *Stenophora* (*Stenophora erratica* Crawley, 1907). He mentioned also that this species does not occur very often in *Gryllus* and that it must be identical with *Stenophora julipusili* Leidy "somewhat altered from being in the wrong host."

The complete description and account of the life-cycle of this gregarine we owe to Watson (1916, pp. 118–120) who discovered the cysts and their method of dehiscence by sporoducts, and thus placed the species in its correct systematic position.

2. *Leidyana gryllorum* (Cuénot) Watson is a parasite of *Gryllus domesticus* L. and is very similar to *L. erratica*. According to Watson (1916, pp. 120–121) "the only difference seems to be in the shape of the protomerit." In *L. erratica* the protomerit "is decidedly conical at the apex" while in this species it is "broadly rounded subspherical in shape; the constriction at the septum is considerably deeper."

---

[1] Also described by Diesing (1859) under the name *Gregarina oviceps*, which was accepted by Watson "as it is the oldest binomial name used."

Adding the new species to the foregoing, the following table shows the chief characters of these three species:

| | *Leidyana erratica* | *Leidyana gryllorum* | *Leidyana tinei* |
|---|---|---|---|
| Sporont . . | solitary | solitary | solitary |
| Maximum length of Sporont . | 500 μ | 420 μ | 300 μ |
| Maximum length of Protomerit . | 80 μ | 84 μ | 40 μ |
| Shape of Protomerit . . | conical | subspherical | subspherical |
| Cysts diameter . | 320 μ | 190–240 μ | 90–110 μ |
| Dehiscence . . | sporoduct | sporoduct | sporoduct |
| Number of sporoducts . . | 1–12 | 3–8 | 2–5 |
| Length of sporoduct . . . | very long (1200 μ) | ? | short (40 μ–50 μ) |
| Spores . . | barrel-shaped | barrel-shaped | barrel-shaped |
| Length of spores | 3–6 μ | 7 μ | 7 μ |
| Host . . . | *Orthoptera:*<br>*Gryllus pennsylvanicus*<br>,, *abbreviatus* | *Orthoptera:*<br>*Gr. domesticus* | *Lepidoptera:*<br>Tineid larvae |
| Geographical distribution . . | America | Europe and America | Europe |

According to the lists of the known gregarines with their hosts which we owe to Minchin (1903) and Watson (1916), the gregarines are very common in the larvae and imagines of Coleoptera (about 100 species of Cephaline gregarines are quoted by Watson), they occur very often in the Orthoptera (35 species) and Neuroptera (20 species). A few gregarines have been described from Diptera, Hemiptera and Thysanura, and no gregarines have been found in the two large orders of holometabolic insects: Lepidoptera and Hymenoptera. The species *Leidyana tinei* here described is the first gregarine recorded as parasitic in Lepidoptera.

The fact that gregarines have not been previously recorded in these two orders cannot be considered as due to lack of observation, since large numbers of these Insects have been dissected for many different purposes. Possibly we can account for the infrequency of their occurrence because of the habits of the larvae of Lepidoptera and Hymenoptera. These are often parasites in other Arthropods (Hymenoptera) or they are gallicolous (Hymenoptera) or phytophagous (Lepidoptera and Hymenoptera) or they live in nests and cells (Hymenoptera). It is interesting to notice that only one of the Coccidia (*Adelea mesnili* Pérez) has been described from Lepidoptera and it was found in the larvae of a Tineid (*Tinea biseliella* Zll.).

On the other hand the *Microsporidia* are often found in the Lepidoptera and Hymenoptera.

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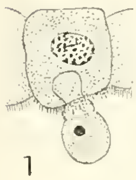
## EXPLANATION OF PLATE IX.

*c.i.*, epithelial cell of intestine of the host. *c.w.*, cyst wall. *d.*, dentomerit. *ex.l.*, external transparent layer of cyst. *ep.*, epimerit. *p.*, protomerit. *sp.*, spores. *spd.*, sporoduct.

Figs. 1, 2, 3, 4, 5 and 6. Different stages of trophozoite, attached to the epithelial cells of the host, or free. × 390.
Fig. 7. A full grown sporont. × 270.
Fig. 8. Sporont with protoplasm showing transverse folds; from a living specimen. × 390.
Fig. 9. A young sporont curved. × 562.
Fig. 10. Trophozoite with protoplasm showing transverse folds. × 390.
Fig. 11. Young cyst from the midgut of the host. × 280.
Fig. 12. Spores.
Fig. 13. Cyst before dehiscence. × 390.
Fig. 14. Cyst during the dehiscence. × 562.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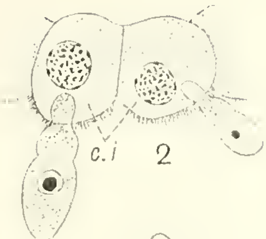

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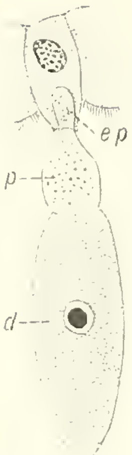

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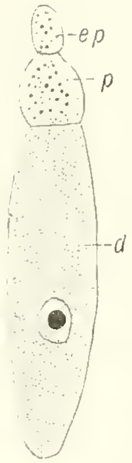

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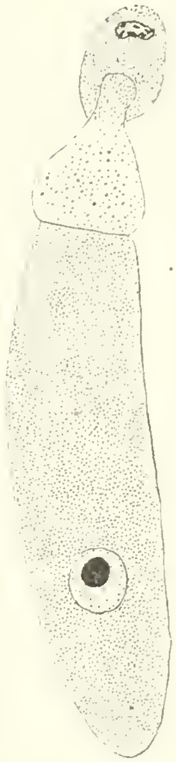
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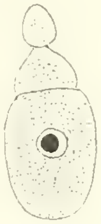
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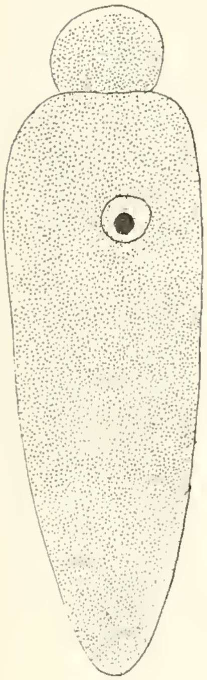
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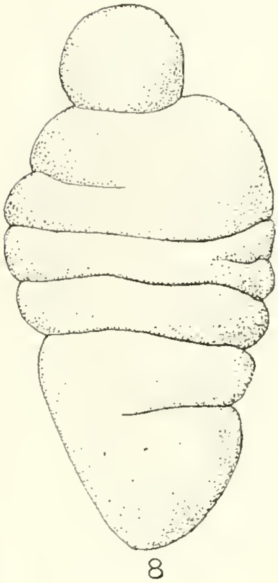
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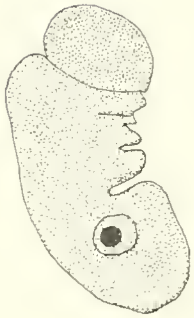
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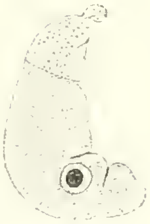
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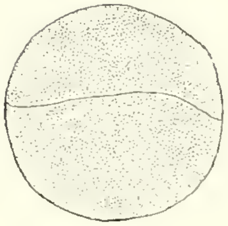
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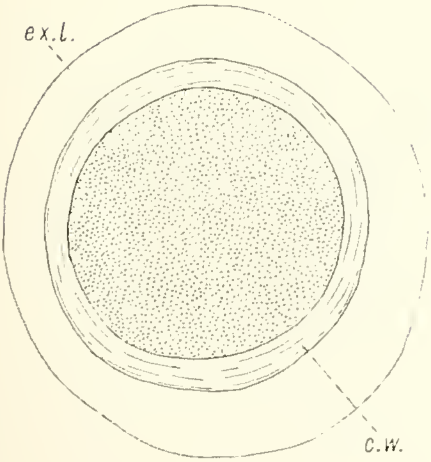

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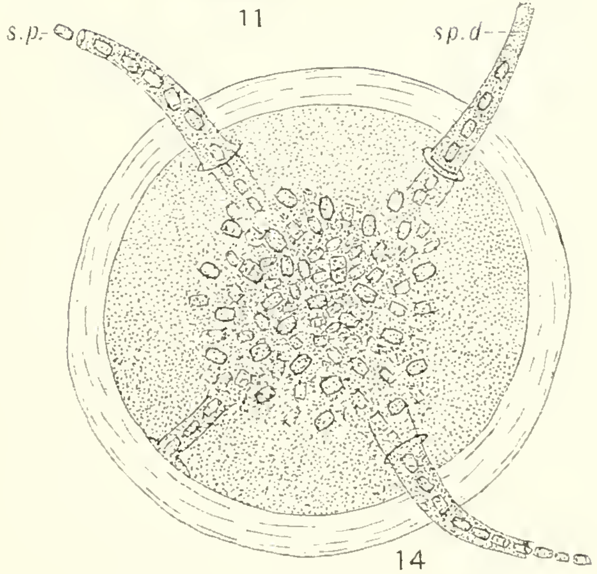

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